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**Evaluation of existing spatial repellents for the control of malaria
vectors in rural Tanzania**

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**Thesis submitted in accordance with the requirements for the
degree of Doctor of Philosophy of the
University of London**

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European Union Seventh Framework Programme FP7 (2007-2013), Grant No
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Declaration

I, Sheila Ogoma Barasa, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

Signature:



Date.....28th March 2015

Abstract

Background: Current malaria vector control programmes rely on insecticides with rapid contact toxicity. However, spatial repellents can also be applied to reduce man-vector contact, with the ultimate goal of reducing malaria transmission.

Objectives and methods: The overall goal of my PhD thesis was to evaluate existing spatial repellents as potential tools for malaria control. This thesis focused on characterizing the effect of pyrethroid spatial repellents on mosquito behaviour indoors and outdoors. Emphasis was placed on the effect on entomological parameters that influence malaria transmission. Experiments were conducted in experimental huts in a malaria endemic village in rural south eastern Tanzania and in a semi-field system against laboratory reared *Anopheles gambiae sensu stricto* mosquitoes.

Results and conclusions: Transfluthrin and Metofluthrin coils and DDT reduced human vector contact through deterrence, irritancy/excito-repellency and blood-feeding inhibition. Pyrethroid coils were shown to cause excitation and increased activity of mosquitoes in the presence of humans. Transfluthrin coils did not hinder attraction of mosquitoes to humans but prevented mosquitoes from biting and blood feeding. This way coils provided area wide protection for up to 15m and prolonged anti-feeding for 12 hours. There was no evidence of Transfluthrin induced repellency (directional movement of mosquitoes away from humans) under outdoor conditions. Locally developed Transfluthrin hessian strips also prevented mosquitoes from biting. This thesis elucidates the mode of action of spatial repellents: spatial repellents reduce human – vector contact

and induce mortality, hence directly affect ma : human biting rate, m : mosquito density and p : mosquito survival which are among the most important parameters of the vectorial capacity of a mosquito population. This information is critical for the development of target product profiles for spatial repellent products. This study shows that spatial repellents may be a suitable complementary option where mosquitoes feed in the early evening and rest outdoors.

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Contributions made by others

Chapter 1: It includes the introduction of the thesis. I did 80% of the work. I drafted the initial draft and Dr. Sarah J. Moore checked and edited it for scientific prowess.

Chapter 2: It includes the systematic literature review. I did 70% of the work. I conducted the literature search and drafted the initial manuscript with help from the senior author Dr. Marta Maia. The co-authors checked the manuscript for grammatical and scientific errors. Suggestions from co-authors were incorporated in the final manuscript.

Chapter 3: I did 70% of the work. I collected data in the field and analyzed the data and drafted the first manuscript with help from my supervisor Dr Sarah J Moore. The final manuscript incorporated suggestions from all co-authors.

Chapter 4: I did 70% of the work. I designed all field and semi-field experiments and drafted study protocols under supervision from Dr. Sarah J. Moore and academic advisors Dr. Lena Lorenz and Dr. Marta Maia. I conducted and supervised all experiments with the help of technicians and my supervisors. I received statistical help from Dr. Lena Lorenz and Dr. Katharina Kreppel. Dr. Sarah J. Moore verified the analysis. I drafted the initial manuscript that was later edited by co-authors. Suggestions from co-authors were incorporated in the final manuscript.

Chapter 5: I did 70% of the work. I designed all field and semi-field experiments and drafted study protocols. Dr. Sarah J. Moore, Dr. Lena Lorenz and Dr. Marta Maia supervised these experiments. I conducted and supervised all experiments with the help of technicians. Dr. Lena Lorenz offered statistical help. Dr. Sarah J. Moore verified the

final analysis. I drafted the initial manuscript that was later edited by co-authors and suggestions from co-authors were incorporated in the final manuscript.

Chapter 6:

I did 80% of the work. I designed the semi-field experiments and drafted the study protocol. I conducted and supervised all experiments with the help of technicians. I analyzed the data and drafted the initial manuscript that was later edited by Dr. Gerry Killeen.

Chapter 7: It includes the general discussion. I did 80% of the work. I drafted the chapter. My supervisor Dr. Sarah J. Moore edited it for scientific prowess and gave suggestions on how to improve it. These suggestions were included in the final draft.

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Acronyms

AIC – Aikakes’ information criterion

DDT – dichlorodiphenyltrichloroethane

ENMoA – entomological modes of action

GEE – generalized estimating equations

GLMM – generalized linear mixed models

HBI – human blood index

HLC – human landing catches

IHI – Ifakara Health Institute

IRS – indoor residual spraying

ITM – insecticide treated materials

ITNs – insecticide treated nets

IVM – integrated vector management

KD – knock down

LLINs – long lasting insecticidal nets

NOEL – no observable effect level

OR – olfactory receptors

ORC – olfactory receptors cells

RVF – rift valley fever

SFS – semi field system

SR – spatial repellents

TPP – target product profile

WHO – World Health Organization

WNV – west nile virus

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CHAPTER ONE

1 General introduction

1.1. Public health importance of malaria in Africa

Malaria burden is generally falling albeit to varying degrees across most regions of the world [1]. From 2000 to 2013 estimated malaria mortality rates dropped by 47% worldwide and by 54% in the World Health Organization Africa region [1]. The success in malaria control is attributed to high coverage of long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) programmes [2, 3] as well as use of effective diagnostic testing, malaria treatment and chemoprevention [1].

Massive scale up of interventions in sub Saharan Africa is attributed to tremendous financing. International financial disbursements to malaria-endemic countries totalled US\$ 2.7 Billion in 2013. [1]. Despite increased funding, the amount available remains below that required to achieve universal access to malaria interventions that would control and eliminate malaria. These are among challenges facing malaria control in sub-Saharan Africa. The development of artemisinin resistance in the Greater Mekong sub-region [4], insecticide resistance in parts of Africa [5], lack of tools that sufficiently reduce vectorial capacity, and the presence of mosquitoes that rest and feed outdoors are factors that may hinder malaria elimination and eradication [6].

1.2. Distribution and bionomics of malaria vectors in Africa

The most important vector of malaria in Africa is *Anopheles gambiae* species complex [7]. It comprises at least six sibling species including: *An. gambiae sensu stricto*, *An. arabiensis*, *An. quadrianulatus*, *An. melas*, *An. merus* and *An. bwambae* [8, 9]. The distribution of these vectors in Africa is shown in Figure 1.1 [10]. Within this complex, *An. gambiae sensu stricto*, *An. arabiensis* and *An. funestus* are the most dominant vector species [11, 12] and are responsible for most of malaria transmission throughout Africa [13]. *Anopheles gambiae s.s.* and *An. funestus* mosquitoes are shown to exhibit anthropophilic (feed on humans) [14-16], endophagic (feed indoors) and endophilic (rest indoors) behaviour [17, 18] and typically feed late at night [19] hence the ability to sustain high malaria transmission in Sub Saharan Africa. Unfortunately, close proximity to humans and indoor insecticides, is believed to have led to selective pressure and development of resistance to pyrethroids followed by resurgence of malaria in Kwa Zulu-Natal, South Africa. *An. arabiensis* mosquitoes exhibit behavioural plasticity that allows them to survive in a range of geographical locations [12]. These mosquitoes are considered to be mainly zoophilic (feed on animals), exophagic (feed outdoors) and exophilic (rest outdoors) [20] but have also been shown to exhibit a range of feeding and resting behaviour showing both anthropophilic and zoophilic behaviour [21]. The plastic behaviour is believed to enhance their adaptability to avoid contact with LLINs and IRS [20]. Studies indicate that *An. gambiae s.s.* mosquitoes that previously predominated in western Kenya [22] and northeastern Tanzania [23] have tremendously decreased in relation to *An. arabiensis*. This change is attributed to the plastic behaviour of *An. arabiensis* that makes them less responsive to intra-domiciliary vector control tools such

as LLINs and IRS. A recent illustration of the distribution of malaria vectors shows predominant distribution of *An. arabiensis* and *An. funestus* mosquitoes in East Africa [24] (Figure 1.2.) compared to Figure 1.1 where *An. gambiae s.s.* were predominant.

1.3. Mosquito control and malaria transmission

The main objective of malaria vector control is reduction of the vectorial capacity.

Vectorial capacity refers to the expected number of new human malaria infections disseminated per human per day, by a mosquito population from a single case, presuming that all vector females feeding on the case become infective [25]. It relates to entomological parameters relevant for malaria transmission including: the density of mosquitoes in relation to man, human biting rate and the daily probability of the survival of vectors [26, 27]. A decrease in the vectorial capacity of mosquitoes leads to a reduction in malaria transmission [25]. The main mosquito control tools include larval source management, use of LLINs and IRS. The distribution of LLINs and high coverage of IRS have led to massive reduction of malaria [28-30]. The main aim of larviciding is to reduce vector density in order to reduce malaria transmission [31]. Efficacy of larviciding depends on location and treatment of all larval habitats. This is challenging in rural areas where larval habitats are many and hard to identify and may be the reason why larviciding is not as effective as other tools [32]. However, larval source management is proven effective against malaria transmission as well as cost effective where mosquito larval habitats are accessible and well defined. IRS is particularly effective where female mosquitoes after feeding, rest inside houses to digest blood meals. Therefore, high coverage of spraying and ensuring that all surfaces are sprayed enhances efficacy of IRS

due to increased contact of mosquitoes with toxic insecticides hence a decrease in survival and population densities. In this respect IRS has successfully reduced malaria transmission where vectors are endophilic [30] but may be ineffective against exophilic mosquitoes. LLINs massively reduce malaria because they affect different stages of the mosquito life cycle hence lead to more gains [33, 34]. They reduce the mosquito lifespan, lengthen the cycle and prevent biting through the killing and repellent actions [33]. However, efficacy is highest where mosquitoes are zoophagic because mosquitoes that are prevented from feeding can be diverted to non-malaria hosts [35]. Other control measures such as improving housing have also been shown to reduce malaria transmission [36, 37] through reduction of indoor densities of mosquitoes and human - vector contact [38, 39].

In order to achieve maximum benefits from vector control interventions, it is necessary to consider the distribution and behaviour of mosquitoes as well as the level endemicity of malaria before implementation. For instance, intra-domiciliary tools are effective where mosquitoes are anthropophilic, endophagic and endophilic but less effective where mosquitoes are exophagic and exophilic [40]. These underscore the need for additional vector control tools that can protect people in the early evening and when they are outdoors. This thesis focuses on the use of spatial repellents as complementary tools for malaria control. Spatial repellents are chemicals that work at a distance in the vapour phase [61] causing mosquitoes to sit apart from the source of the chemical [62]. Spatial repellents render a specific area mosquito free by preventing landing or biting within that area [63].

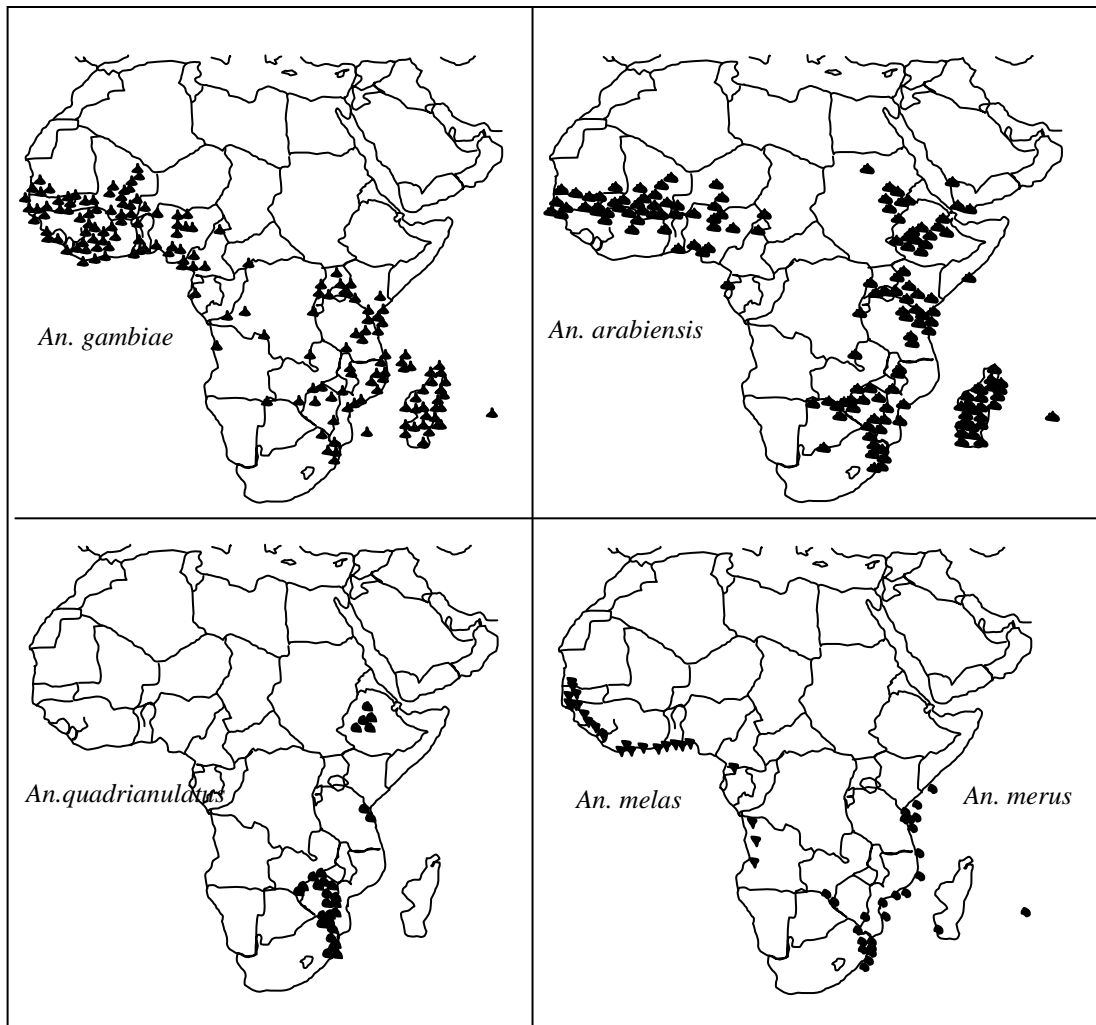


Figure.1.1: Map of Africa showing distribution of *Anopheles gambiae* species complex mosquitoes. (These maps were produced in 2000). Source: [10].

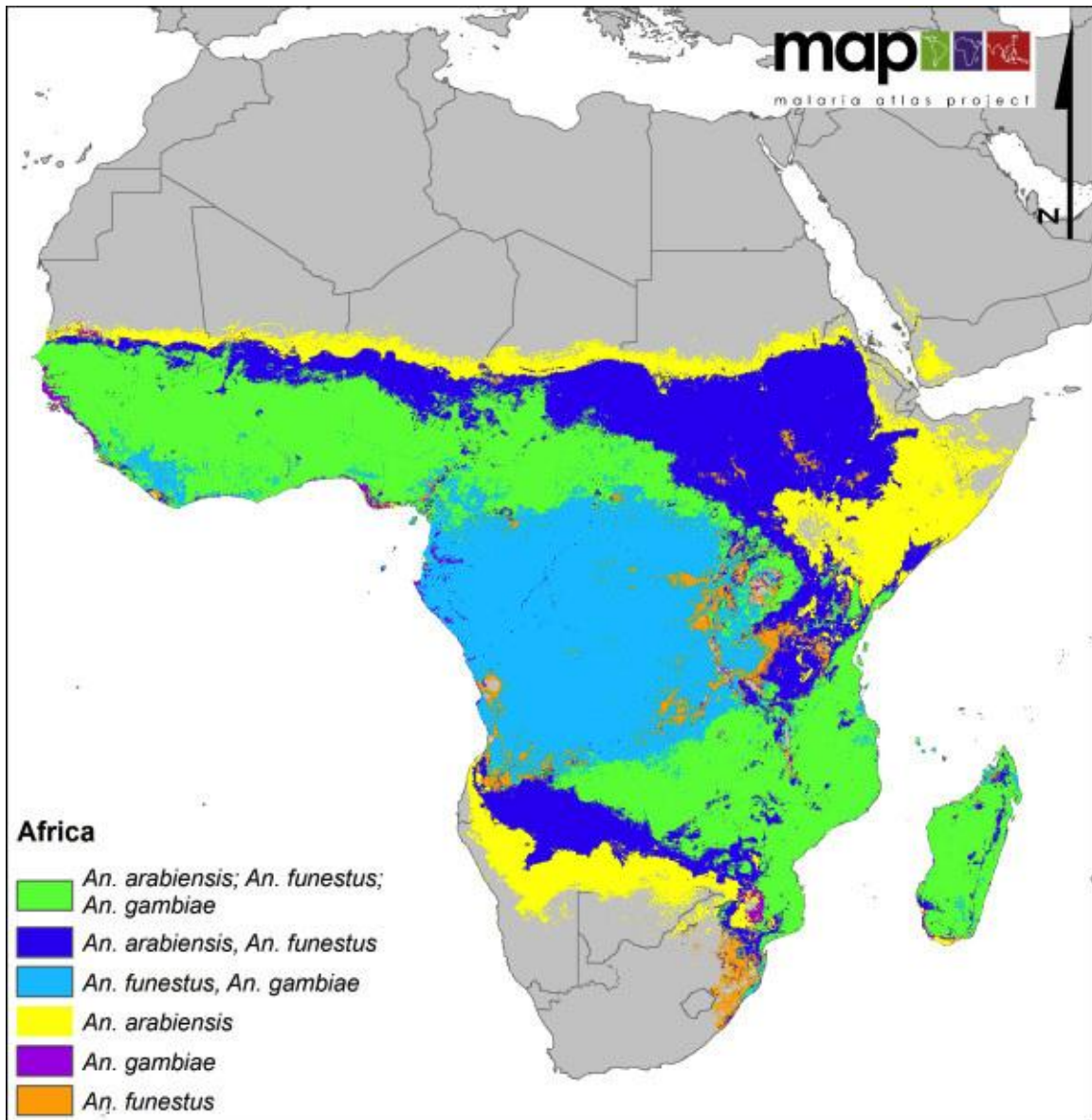


Figure. 1.2. A regional map showing the distribution of the three most dominant malaria vectors in Africa. (This map was produced in 2012) Source: [24].

1.4. Statement of problem

The World Health Organization recommends full coverage of Long Lasting Insecticidal Nets (LLINs) for all people at risk of malaria [41], and Indoor Residual Spraying (IRS) for low and moderate transmission areas in order to reduce seasonal annual malaria transmission [42]. Recent massive scale up of LLINs and IRS has successfully reduced malaria throughout sub Saharan Africa including Tanzania [2, 3]. Despite these successes, elimination and eradication remain challenging for most countries in Africa [6]. The main goal for vector control is to reduce vectorial capacity to levels that prevent transmission of parasites. Existing vector control tools are failing to elicit complete malaria control due to factors including: development of insecticide resistance and the presence of mosquitoes that rest and feed outdoors [6].

Use of LLINs and IRS targets night biting, indoor feeding (endophagic) and indoor resting mosquitoes (endophilic), which are primarily anthropophilic (feed on man). High coverage of LLINs and IRS has successfully reduced these mosquitoes in most areas in Africa [22, 43, 44]. Unfortunately, success of indoor mosquito control means that there is a competitive advantage for those species and sub-species that feed when hosts are still active outdoors and they are able to maintain transmission, albeit at lower levels [40]. LLINs prevent blood feeding and therefore disrupt the feeding process of mosquitoes, increasing the length of the oviposition cycle of the overall population. This mechanism might explain the immediate change in biting cycles of several species such as has been reported for *An. farauti* and *An. koliensis* in Papua New Guinea [45]. Other studies also

report complete species shift to those that are able to feed and rest outdoors [22, 43, 44] and the appearance of cryptic species that have increased outdoor activity [46]. In addition, reduced malaria transmission is accompanied by heterogeneous transmission that has created transmission hot spots that remain highly malaria endemic, despite existing control programmes [47]. This indicates that apart from new control tools there is need to identify and target hot spots that continue to maintain transmission [47]. So far, there has been no deliberate control strategy geared towards early evening or outdoor biting and resting mosquitoes in sub-Saharan Africa, although in Greater Mekong sub region outdoor malaria transmission is well recognized [48, 49]. In order to eliminate and eventually eradicate malaria, efforts should be put into developing tools that target outdoor biting mosquitoes. Tools that protect people when they are not protected by LLINs and IRS are topical repellents. Topical repellents protect people from early – biting mosquitoes and the effect on reduced malaria transmission has been reported [50-52]. However, most topical repellents provide an average of 4 -10 hours protection [53] hence the development of long lasting formulations that confer maximum protection is necessary. Furthermore, efficacy of topical repellents is highly dependent on regular compliance by users. Studies indicate that when there is inconsistent compliance, mosquitoes are likely to be diverted between users and non-users. This might undermine control efforts due to increased diversion of infectious mosquitoes to unprotected people, who are potentially the poorest members of a population (unable to afford repellents), hence most vulnerable to the effects of malaria [54]. Efficacy of other interventions such as LLINs is also attenuated by lack of compliance by the community [55, 56].

Behavioural, change is needed to make compliance a daily occurrence. It is widely recognized that interventions requiring minimal compliance by users are needed.

One such method, to protect people at times when they are near to the home but not yet under their bednets are Spatial Repellents (SR) [57-60]. Spatial repellents are chemicals that work at a distance in the vapour phase [61] causing mosquitoes to either fly away from the source of the chemical [62] or mask human odours and prevent attraction. Spatial repellents render a specific area mosquito free by preventing landing or biting within that area [63]. They are also referred to as area repellents. Examples of SR include vaporizing mats, candles, mosquito coils and passive emanators [64].

There is need to develop passive means of dispensing SR that provide area wide protection especially outdoors and extend protection to the whole household rather than to one person. Mosquito coils are commonly used in sub-Saharan Africa and their efficacy is well documented [64]. However, coils burn out after 6-8 hours, hence requiring replacement and therefore may end up being expensive and unaffordable. In addition, coils produce smoke that may be undesirable. To solve this problem, paper strips and plastic impregnated with the volatile pyrethroid Metofluthrin have been developed [59, 65-67]. The active ingredient evaporates at ambient temperature without the need for electricity or heating. This suggests that they can be used anywhere and makes them suitable for developing regions where there is no electricity. Like coils, emanators reduce landing and biting mosquitoes and have been shown to be effective against indoor and outdoor biting mosquitoes [59, 68, 69]. Efficacy of these emanators lasts a few weeks, and thus there is a need to develop novel passive ways of dispensing

volatile chemicals that last longer: preferably as long as a malaria transmission season.

There is need to generate more data on the entomological mode of action i.e. the way a vector control intervention works preventing disease transmission by killing, repelling or inhibiting reproduction of target insects [70] of spatial repellents that can be linked with the epidemiological modes of action in order to understand the impact of SR on malaria transmission.

The overall goal of this thesis is to determine the range of mosquito behaviours elicited by SR that minimize human - vector contact and consequently reduce malaria transmission. The SR selected for evaluation in this thesis are: Transfluthrin and Metofluthrin mosquito coils and DDT used as IRS.

Metofluthrin and Transfluthrin compounds are highly volatile at ambient temperature; and hence are good candidates for passive emanators. However, the mode of action is not fully understood. This study focuses on determining the mode of action during indoor and outdoor use.

Firstly, coils were selected because they have been extensively studied. [64]. Secondly, coils burn uniformly for 6-8 hours thus the release rate of the active ingredient is likely to be constant over time. Thirdly, coils are widely used for protection against mosquito bites and have high user acceptability [71]. This makes them suitable for use in experimental studies involving humans who are easily convinced to use them unlike when the product being tested is new. The mosquito coils used in the study have undergone rigorous safety testing and were donated by a reputable Personal Products supplier: SC Johnson. We were therefore sure that they were of the correct concentration and free of contaminants that could cause respiratory complications.

Mosquito coils were compared to DDT used as IRS because its mode of action as SR and the effect on malaria transmission are well documented [72, 73]. DDT was sprayed on palm woven mats. This enabled rotation between huts alongside other treatments and reduced locational bias that could have arisen from experimental huts.

1.5. Hypotheses

It is hypothesized that airborne pyrethroids 1) induce mosquitoes to move away from the highest concentration of the molecule to an area of lower concentration [74], 2) interfere with host detection [72] and blood feeding behaviour of mosquitoes [65, 69, 75] and also 3) prevent mosquitoes from flying through sub-lethal incapacitation [76-78]. This study aims at characterizing the effect of pyrethroid based spatial repellents on mosquito behavior that reduces their vectorial capacity. The effect of DDT, Metofluthrin coils and Transfluthrin coils on house entry and exiting, host seeking, blood feeding, mosquito fertility and survival of the Afro Tropical malaria vectors *An. gambiae s.s.* and *An. arabiensis* were measured in experimental huts and the semi-field system.

1.6. Specific objectives

1. To perform an in-depth literature review, on existing studies of spatial repellents with emphasis on mosquito coils and passive emanators that are suitable for use in rural areas

2. To develop and standardize an assay for evaluation of the modes of action of volatile pyrethroids: Metofluthrin and Transfluthrin against outdoor biting mosquitoes
3. To measure the effect of airborne pyrethroids: Metofluthrin and Transfluthrin released by mosquito coils on mosquito behaviours that influence entomological parameters of malaria transmission indoors and outdoors
4. To determine the effect of volatile Transfluthrin in coils on the host seeking and blood-feeding behaviour of mosquitoes
5. To evaluate an appropriate alternative low-cost, passive format of dispensing volatile Transfluthrin to protect humans against outdoor biting mosquitoes suitable for use by the poor people in rural sub-Saharan Africa

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CHAPTER TWO

2 Literature review

A systematic review of mosquito coils and passive emanators: defining recommendations for spatial repellent testing methodologies of spatially acting pyrethroids

2.1. Abstract

Mosquito coils, vaporizer mats and emanators confer protection against mosquito bites through the spatial action of emanated vapour or airborne pyrethroid particles. These products dominate the pest control market; therefore, it is vital to characterize mosquito responses elicited by the chemical actives and their potential for disease prevention. The aim of this review was to determine effects of mosquito coils and emanators on mosquito responses that reduce human-vector contact and to propose scientific consensus on terminologies and methodologies used for evaluation of product formats that could contain spatial chemical actives, including indoor residual spraying (IRS), long lasting insecticide treated nets (LLINs) and insecticide treated materials (ITMs). PubMed, (National Centre for Biotechnology Information (NCBI), U.S. National Library of Medicine, NIH), MEDLINE, LILAC, Cochrane library, IBECS and Armed Forces Pest Management Board Literature Retrieval System search engines were used to identify

studies of pyrethroid based coils and emanators with key-words “Mosquito coils” “Mosquito emanators” and “Spatial repellents”. It was concluded that there is need to improve statistical reporting of studies, and reach consensus in the methodologies and terminologies used through standardized testing guidelines. Despite differing evaluation methodologies, data showed that coils and emanators induce mortality, deterrence, repellency as well as reduce the ability of mosquitoes to feed on humans. Available data on efficacy outdoors, dose–response relationships and effective distance of coils and emanators is inadequate for developing a target product profile (TPP), which will be required for such chemicals before optimized implementation can occur for maximum benefits in disease control.

Keywords: Spatial repellents, Pyrethroids, Coils, Passive emanators, Mosquito responses

2.2. Review

Currently, control of malaria vectors relies almost entirely on indoor residual-spraying (IRS) and Long Lasting Insecticidal Nets (LLINs) [1]. These vector control tools have successfully reduced mosquito population densities and malaria by targeting indoor-feeding (endophagic) and indoor-resting (endophilic) mosquitoes [2]. The most successful IRS chemical active used to date is DDT, which, in addition to killing mosquitoes, also reduces indoor mosquito densities consequently reducing malaria transmission [3-6].

Literature shows that much of the success of DDT is due to excito-repellency [4, 5]. An excito-repellent is defined as a chemical that causes insects to make undirected movements that set them apart from insecticides [7]. Excito-repellency results from insect's physical contact with chemicals on treated surfaces or with vapour particles at a distance [8, 9]. It has been demonstrated that volatile DDT can induce neural excitement in insects [10] and importantly, it was observed that insects exposed to sub-lethal concentrations of DDT move towards the light explaining why mosquitoes are likely to quickly leave a sprayed dwelling [11]. Excito-repellency was also originally seen as a beneficial feature of pyrethroid treated bednets to reduce the probability of mosquitoes developing resistance to insecticides through lower contact with insecticides [12]. It is known that DDT and pyrethroids act on the voltage-gated sodium channel proteins found in insect nerve cell membranes, disrupting transmission of nerve impulses thereby causing mortality [13]. Cross resistance between DDT and pyrethroids is conferred by point mutations on the voltage gated sodium channel in mosquitoes indicating a common mode of toxic action for these insecticides on mosquitoes [14]. Mechanisms underlying host-seeking and feeding behaviours of mosquitoes are largely unknown and have been the topic of current investigations. It is known that sublethal exposure to both pyrethroids and DDT has a differing effect on insect feeding responses: pyrethroids inhibit responses to attractants while DDT increases neural sensitivity to attractive sources [15, 16]. New advancements in the field of neurobiology have demonstrated that perception of chemicals in the environment by insects begins when compounds activate ionotropic receptors, gustatory receptors and olfactory receptors (ORs) located on the dendritic surface of chemosensory neurons of the olfactory receptor cells (ORCs) housed in a head

appendage (e.g. antenna or palp) [17]. ORs recognize biologically meaningful chemical ligands, and shape responses of olfactory sensory neurons (OSNs), thus regulating many behaviours including repellency.

Repellents either activate or inhibit action of ORs interfering with the host-seeking behaviour of mosquitoes, resulting in repellency or anti-feeding [18]. A repellent pyrethroid has been shown to disrupt insect behaviour not through targeting the voltage gated sodium channel but instead inhibits the response of odorant receptors (ORs) to attractants in a similar way to para-menthane 3,8 diol and nepetalactone [18]. Repellency is a characteristic of personal protection tools such as mosquito coils, liquid vaporizers, vaporizer mats and ambient emanators [19]. These tools have been extensively studied yet they have not been promoted as formal methods for mosquito control. In 2006 the consumer market for pesticides was about \$8.4 billion, with expected double-digit annual growth mainly due to rising income levels in several developing-world markets, notably China [20]. By far the most popular segment was aerosols, at \$3.6 billion, followed by topical repellents, powders, and gels at \$2 billion. The smaller segments of mats and vaporizers accounted for \$1.6 billion and coils for \$1 billion [20]. These products are already widely used and would therefore be expected to have community uptake if they were introduced as a formal means of disease control in an integrated vector management (IVM) strategy.

In addition, due to increased need for effective vector control tools, to combat residual outdoor-biting and resting mosquitoes [21], it is timely to review studies of mosquito coils and emanators. This will enable better understanding of their mode of action and hence gain useful knowledge for development of effective spatially acting chemical

products that can be used outdoors hence complement LLINs and IRS for integration into a malaria elimination strategy [22].

The main active ingredients recommended by the World Health Organization (WHO) for use in the vapour phase all belong to the pyrethroid chemical class. The most commonly used format; mosquito coils are cheap and effective but produce smoke [23] which is undesirable. Vaporizer mats are an alternative to coils. The mats contain embedded repellent active ingredients that are volatilised using an electric heating element. The need for electricity can increase product costs making them inappropriate for some rural and urban settings in low or middle-income countries.

Recently, other delivery formats that do not require heating or combustion have been developed. These are commonly known as emanators and are composed of insecticides impregnated on substrates such as paper, plastic or agarose-based gels [24, 25]. Unlike coils and mats, emanators function through passive evaporation of chemical actives. These chemicals are less polar and have lower vapour pressure than conventional pyrethroids hence evaporate at ambient temperature without the need for an external source of energy. Examples of these insecticides include metofluthrin and transfluthrin.

The aim of this review was to determine effects of mosquito coils and emanators on mosquito responses that reduce human-vector contact and to propose scientific consensus on terminologies and methodologies used for evaluation of product formats that could contain spatial repellents including IRS, LLINs and insecticide treated materials (ITMs).

This review was conducted in accordance with PRISMA (Preferred Reporting Items for

Systematic Reviews and Meta-Analyses) guidelines [26]. PubMed, (National Centre for Biotechnology Information (NCBI), National Library of Medicine, NIH), MEDLINE, LILACS, Cochrane library, IBECs and Armed Forces Pest Management Board Literature Retrieval System were searched systematically for both field and laboratory studies that included pyrethroid based coils and/or emanators using the English key-words “Mosquito coils”, “Mosquito emanators” and “Spatial repellents”, between January and November 2011. In addition to journal articles, we searched reference lists of identified papers. We also checked the System for Information on Grey Literature in Europe (SIGLE) for unpublished data from sources such as conference proceedings and abstracts in an attempt to avoid the so called top drawer effect where only positive findings are published. The last search was conducted on 21st September 2012. We were confident that the search engines we used provided almost all relevant studies of interest. Data were extracted from selected articles that met all study criteria using a standardized spreadsheet. The information collected included first author, year of publication, methods and design, active ingredient, dose, mosquito species, sample size, description of the control, testing conditions (experimental huts, rooms, chambers or cylinders) and the outcome measures reported with any available statistical information.

2.3. Inclusion and exclusion criteria

All publications evaluating coils and/or emanators were reviewed. However, to facilitate comparison of bioefficacy of different active ingredients across studies, the following selection scheme was employed (Figure 2.1): (i) laboratory and field studies were

reviewed separately; (ii) only laboratory and field studies that quantified mosquito responses including biting/feeding inhibition of mosquitoes, knock-down time and percentage mortality 24 hours post-exposure to insecticides, deterrence, repellency or irritancy of insecticides were included; (iii) studies where the dose of active ingredient was not indicated were excluded, (iv) all studies where coils contained a mixture of insecticides or additives were excluded.

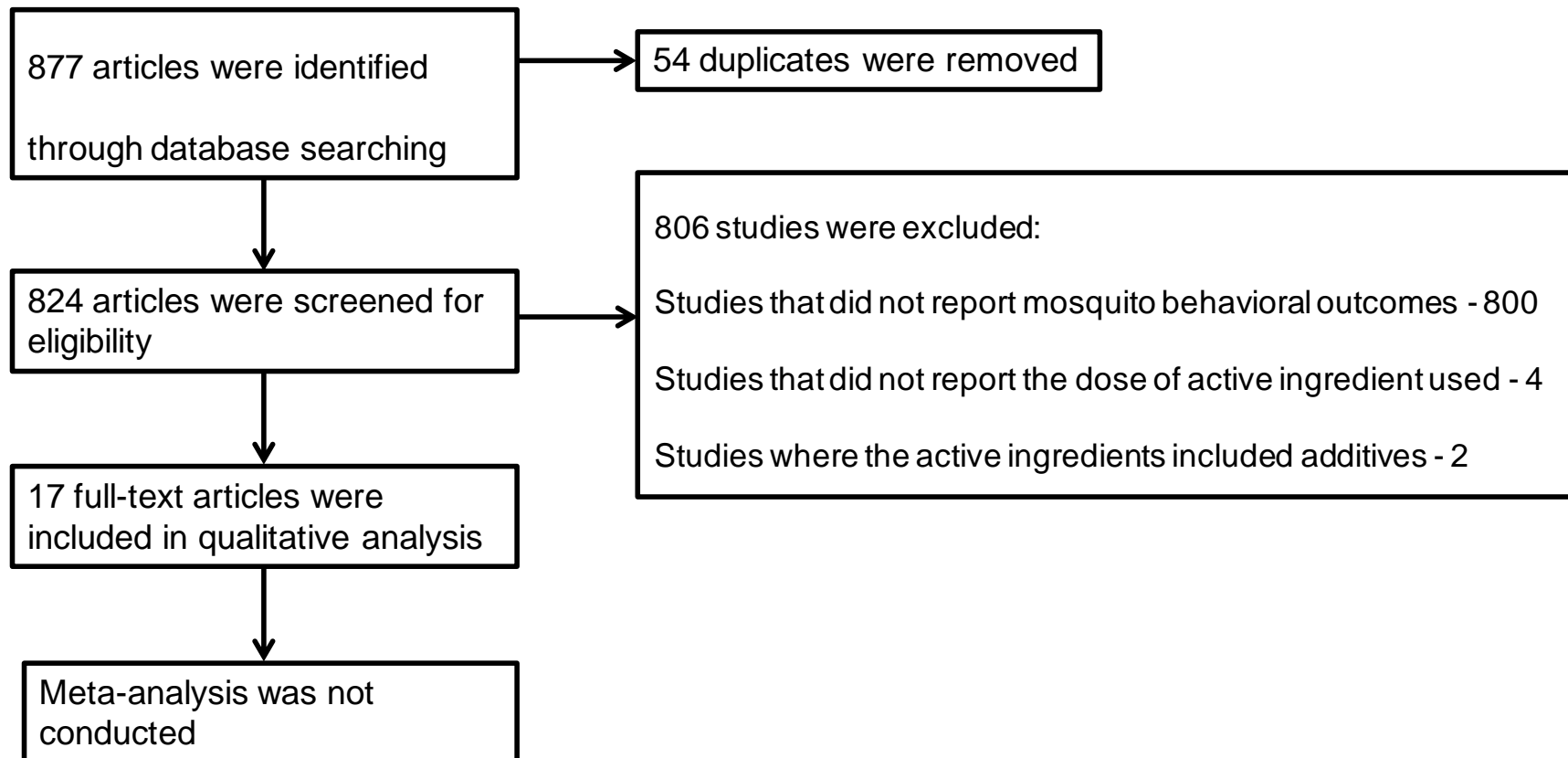


Figure 2.1: A flow diagram of the selection procedure of articles included in the systematic review

2.4. Summaries of reported mosquito responses to coils and emanators, and suggestions for harmonization of terminologies

Several investigators report a number of mosquito responses to airborne insecticide particles. These responses are classified into measurable indicators namely: deterrence, repellency and irritancy, biting/feeding inhibition, knock-down and mortality. Scientific discussions differentiate between mechanisms in mosquitoes leading to responses elicited in the presence of chemical actives and the outcomes quantified [4, 7, 8, 11], this review is restricted to measured behavioural endpoints or consequences and not possible mechanisms causing them.

All studies identified and included in the review evaluated formulated/optimized emanators and coils. It should be noted that comparison of pyrethrins to metofluthrin emanators is only appropriate if both actives were formulated or both were neat material (unoptimized) as effects on volatilization and longevity (among other chemical properties) will be different and bias analyses. This holds true even for comparing results of the same active ingredient.

2.4.1. Deterrence

Airborne insecticide particles present inside and around houses create a chemical barrier that prevents mosquitoes from entering [27]. Deterrence has been measured in the field by comparing the number of mosquitoes entering houses with insecticides and those without. Coils containing pyrethrins deter between

45% and 80% mosquitoes (Table 2.1) and 200mg optimized metofluthrin emanators reduce mosquitoes by > 80% within the first 4 weeks of treatment [28]. However, results from these studies cannot be generalized for other spatial repellent compounds due to potential differences in product formulation i.e., optimized components for release and retention. Only one study measured dose-dependent effects of pyrethrum coils [29] and showed no correlation between the proportion of mosquitoes deterred and the dose of pyrethrum (Table 2.1). Reduced indoor density of mosquitoes in insecticide treated houses could be due to the spatial action of chemical actives that interfere with the host seeking process of mosquitoes making the houses less attractive even when humans are present. In addition, mosquitoes entering treated houses are prevented from feeding. Such observations warrant further investigations of spatially acting chemicals.

Table 2.1.: Mosquito behavioural reactions induced by burning pyrethrum coils in experimental huts

| Dose of pyrethrum (w/w %) | Vector | Feeding inhibition (%) | Non-Contact irritancy (%) | Deterrence (%) | Mortality (%) |
|---------------------------|-----------------------------------------------|------------------------|---------------------------|----------------|---------------|
| 0.10 | <i>Anopheles gambiae</i> Gillies ^a | 54 | 82 | 51 | 16 |
| 0.10 | <i>Culex fatigans</i> | 26 | 58 | 64 | 4 |
| 0.10 | <i>Mansonia uniformis</i> | 24 | 93 | 45 | 3 |
| 0.50 | <i>Anopheles gambiae</i> Gillies | 60 | 87 | 58 | 15 |
| 0.50 | <i>Culex fatigans</i> | 46 | 67 | 51 | 7 |
| 0.50 | <i>Mansonia uniformis</i> | 69 | 87 | 58 | 15 |

^aThe sub species of *Anopheles gambiae* Gillies was not specified. These data is from Smith *et al* 1972 [29].

2.4.2. Repellency and irritancy

Repellency was originally defined to refer to the distribution of insects/mosquitoes on chemically treated surfaces compared to untreated surfaces [11]. This description considers the end result of the effect of chemicals and does not account for a series of preceding behaviours exhibited by mosquitoes that lead to the final outcome. Therefore, this definition was refined to refer to movement of mosquitoes away from a source to which they would otherwise be attracted [30]. Dethier described two kinds of behaviour causing insects to sit apart from insecticide treated surfaces: [7] “taxis”: - immediate directional reaction, resulting in movement away from a treated surface and; 2) “orthokinesis”: - increased undirected activity after contact with insecticides. Both reactions reduce mosquitoes on treated surfaces [7, 8]. These terms have been developed further to include “contact irritancy” where mosquitoes make oriented movement away from a chemical source after physical contact with insecticide treated surfaces [3, 4] and “non-contact irritancy”, where mosquitoes move away when exposed to vapour insecticide particles usually operating at a distance. This has also been described as “spatial repellency” [4, 31], or “area repellency” [32] or “non-contact disengagement” [8]. Non-contact irritancy, spatial repellency and non-contact disengagement all describe behavioural endpoints resulting from exposure to chemical emanations from coils and emanators. For purposes of clarity we propose that spatial repellency should be used as a general term to refer to the sum of mosquito behaviours produced by airborne chemicals that result in mosquitoes sitting apart from a source of stimulation [8].

“Non-contact irritancy” was measured in the field using local houses or experimental huts

fitted with exit- and entry-traps [29, 33-35] by comparing the proportion of mosquitoes exiting untreated and treated structures. Using this approach, studies have demonstrated an increased proportion of mosquitoes that exit earlier from huts with burning coils compared to huts that do not have coils [29]. There was a positive correlation between the proportion of mosquitoes exiting huts and the concentration of the active ingredient [29]. This indicates that the magnitude of irritancy might be dose-dependent [31]. An effective way of measuring “non-contact irritancy” is by releasing laboratory-reared mosquitoes inside experimental huts [4] and observing how fast they leave treated huts compared to control huts. This field data demonstrated good correlation with laboratory data from a high-throughput screening system (HiTSS) developed for evaluating behavioural mode of action of active ingredients [4].

2.4.3. Biting/feeding inhibition

Feeding or biting inhibition is where mosquitoes are prevented from biting or feeding on humans. Coils reduce the biting rate of mosquitoes (Table 2.1). Small amounts of insecticides [36] or repellents have been shown to interfere with the host-seeking process of disease vectors [37, 38]. Sometimes mosquitoes land on the host but do not feed in the presence of repellent actives [39]. Therefore, the act of feeding (probing) should be quantified rather than landing rate. Only one study displayed an increase in the proportion of mosquitoes inhibited from feeding when the dose was increased [29]. In some cases even the smoke which does not contain chemicals reduces biting rate significantly compared to

controls where coils are not used [40]. This warrants the need to conduct more studies with different doses of spatial chemical actives and to generate dose–response curves which will enhance better understanding of the mode of action.

The most accurate and representative method to measure feeding inhibition is through human landing catch (HLC) [41]. Some studies use guinea-pigs as bait [42], which are not proxy indicators for man. A study comparing biting inhibition on guinea pigs and man indicated that guinea pigs underestimated reduction in biting inhibition [42]. This is because guinea pigs do not produce sufficient heat, moisture and carbon dioxide and have a different composition of head space kairomones hence do not attract anthropophilic mosquitoes as much as humans. We propose conducting HLC evaluations inside semi-field systems (SFS) using laboratory reared disease-free mosquitoes to reflect the end use of spatial repellents, while protecting participants from potential exposure to disease carrying mosquitoes.

2.4.4. Knock-down and mortality

Knocked-down (KD) is the incapacitation of mosquitoes after contact with a sub-lethal dose of insecticide [43] resulting in the inability of the insect to maintain normal posture or fly.

High concentrations of pyrethrins induce faster KD50 (within 3–5 minutes of exposure) followed by high mortality rate while low concentrations induce slower KD50 (more than 10 minutes after exposure) indicating a dose–response relationship (Table 2.2). It is also important to note that coils induce up to 95% mortality in laboratory-assays compared to very low levels observed in field-assays (3%–16%) (Table 2.2). This is attributed to volume

and/or ventilation limitations that may occur in some laboratory subsequently assay spaces, which reduce insecticide dispersion consequently increasing relative insecticide concentration.

The relationship between KD50 and mortality of mosquitoes exposed to different pyrethroid mosquito coils is presented in Figure 2.2; 2.3 and 2.4.

Table 2.2. Knock-down time and mortality of mosquitoes after exposure to smoke from mosquito coils impregnated with different doses of Allethrin, d- allethrin, d,d-T-plarethrin, dl,d-T80 allethrin, d-trans allethrin, Esbiothrin, Pyrethrin, S-d- t –allethrin, and Terallethrin pyrethroids.

| Dose (w/w %) | Vector | Mortality (%) | Knock-down (KT50 minutes) | Method |
|-------------------------|----------------------------------|----------------------|--------------------------------------|----------------------------|
| 0.60 | <i>Culex pipiens pallens</i> | 0.12 | 5.10 | 70 cm ³ Chamber |
| 0.60 | <i>Stegomyia (Aedes) aegypti</i> | 0.72 | 3.10 | 70 cm ³ Chamber |
| 0.60 | <i>Anopheles stephensi</i> | 0.81 | 3.20 | 70 cm ³ Chamber |
| 0.50 | <i>Anopheles stephensi</i> | 33.00 | 9.50 | 25m ³ room |
| 0.25 | <i>Anopheles stephensi</i> | 38.00 | 11.10 | 25m ³ room |
| 0.20 | <i>Anopheles stephensi</i> | 25.00 | 11.30 | 25m ³ room |
| 0.15 | <i>Anopheles stephensi</i> | 32.00 | 14.50 | 25m ³ room |
| 0.50 | <i>Stegomyia (Aedes) aegypti</i> | 88.00 | 14.90 | 25m ³ room |
| 0.25 | <i>Stegomyia (Aedes) aegypti</i> | 70.00 | 24.80 | 25m ³ room |
| 0.20 | <i>Stegomyia (Aedes) aegypti</i> | 54.00 | 29.00 | 25m ³ room |
| 0.20 | <i>Anopheles stephensi</i> | 49.00 | 4.50 | 500 mm by 300 mm cylinder |
| 0.15 | <i>Anopheles stephensi</i> | 49.00 | 4.90 | 500 mm by 300 mm cylinder |
| 0.10 | <i>Anopheles stephensi</i> | 42.00 | 5.50 | 500 mm by 300 mm cylinder |

| | | | | |
|------|---------------------------------------|-------|--------|------------------------------------|
| 0.05 | <i>Anopheles stephensi</i> | 32.00 | 6.80 | 500 mm by 300 mm cylinder |
| 0.20 | <i>Stegomyia (Aedes) aegypti</i> | 95.00 | 6.20 | 500 mm by 300 mm cylinder |
| 0.15 | <i>Stegomyia (Aedes) aegypti</i> | 73.00 | 7.50 | 500 mm by 300 mm cylinder |
| 0.10 | <i>Stegomyia (Aedes) aegypti</i> | 54.00 | 10.00 | 500 mm by 300 mm cylinder |
| 0.05 | <i>Stegomyia (Aedes) aegypti</i> | 26.00 | 16.00 | 500 mm by 300 mm cylinder |
| 0.30 | <i>Culex pipiens pallens</i> | 0.15 | 3.80 | 70 cm ³ Chamber |
| 2.00 | <i>Stegomyia (Aedes) aegypti</i> | 0.32 | 1.57 | 2m ³ Peet-Grady chamber |
| 2.00 | <i>Culex quinquefasciatus</i> | 0.49 | 0.98 | 2m ³ Peet-Grady chamber |
| 2.00 | <i>Anopheles stephensi</i> | 0.67 | 1.94 | 2m ³ Peet-Grady chamber |
| 0.30 | <i>Anopheles stephensi</i> | 0.81 | 2.40 | 70 cm ³ Chamber |
| 0.30 | <i>Stegomyia (Aedes) aegypti</i> | 0.84 | 2.40 | 70 cm ³ Chamber |
| 0.10 | <i>Stegomyia (Aedes) aegypti</i> | 0.22 | 171.00 | 25m ³ room |
| 0.10 | <i>Stegomyia (Aedes) aegypti</i> | 0.24 | 120.00 | 25m ³ room |
| 0.10 | <i>Culex pipiens quinquefasciatus</i> | 0.25 | 108.00 | 25m ³ room |
| 0.15 | <i>Stegomyia (Aedes) aegypti</i> | 0.25 | 140.00 | 25m ³ room |
| 0.20 | <i>Stegomyia (Aedes) aegypti</i> | 0.28 | 130.00 | 25m ³ room |
| 0.10 | <i>Culex pipiens quinquefasciatus</i> | 0.30 | 55.00 | 25m ³ room |

| | | | | |
|------|---------------------------------------|------|--------|-----------------------|
| 0.15 | <i>Stegomyia (Aedes) aegypti</i> | 0.30 | 100.00 | 25m ³ room |
| 0.20 | <i>Stegomyia (Aedes) aegypti</i> | 0.30 | 85.00 | 25m ³ room |
| 0.10 | <i>Culex pipiens pallens</i> | 0.36 | 20.60 | 25m ³ room |
| 0.15 | <i>Culex pipiens pallens</i> | 0.39 | 14.00 | 25m ³ room |
| 0.15 | <i>Culex pipiens quinquefasciatus</i> | 0.47 | 100.00 | 25m ³ room |
| 0.20 | <i>Culex pipiens quinquefasciatus</i> | 0.50 | 63.00 | 25m ³ room |
| 0.10 | <i>Culex pipiens pallens</i> | 0.51 | 14.50 | 25m ³ room |
| 0.15 | <i>Culex pipiens pallens</i> | 0.53 | 11.40 | 25m ³ room |
| 0.15 | <i>Culex pipiens quinquefasciatus</i> | 0.55 | 42.00 | 25m ³ room |
| 0.20 | <i>Culex pipiens pallens</i> | 0.67 | 13.10 | 25m ³ room |
| 0.20 | <i>Culex pipiens quinquefasciatus</i> | 0.71 | 24.00 | 25m ³ room |
| 0.10 | <i>Anopheles dirus</i> | 0.91 | 8.00 | 25m ³ room |
| 0.10 | <i>Anopheles dirus</i> | 0.91 | 8.00 | 25m ³ room |
| 0.20 | <i>Culex pipiens pallens</i> | 0.92 | 10.30 | 25m ³ room |
| 0.20 | <i>Anopheles dirus</i> | 1.00 | 8.10 | 25m ³ room |
| 0.27 | <i>Culex pipiens quinquefasciatus</i> | 0.04 | 196.00 | 25m ³ room |
| 0.27 | <i>Stegomyia (Aedes) aegypti</i> | 0.15 | 361.00 | 25m ³ room |

| | | | | |
|------|---------------------------------------|------|--------|------------------------------------|
| 0.27 | <i>Culex pipiens pallens</i> | 0.20 | 28.30 | 25m ³ room |
| 0.27 | <i>Stegomyia (Aedes) aegypti</i> | 0.21 | 174.00 | 25m ³ room |
| 0.27 | <i>Culex pipiens pallens</i> | 0.27 | 18.60 | 25m ³ room |
| 0.50 | <i>Culex pipiens pallens</i> | 0.28 | 20.80 | 25m ³ room |
| 0.50 | <i>Stegomyia (Aedes) aegypti</i> | 0.29 | 170.00 | 25m ³ room |
| 0.27 | <i>Culex pipiens quinquefasciatus</i> | 0.35 | 41.00 | 25m ³ room |
| 0.50 | <i>Culex pipiens quinquefasciatus</i> | 0.55 | 72.00 | 25m ³ room |
| 0.27 | <i>Anopheles dirus</i> | 1.00 | 11.10 | 25m ³ room |
| 0.50 | <i>Anopheles dirus</i> | 1.00 | 8.00 | 25m ³ room |
| 0.30 | <i>Culex pipiens pallens</i> | 0.18 | 3.90 | 70 cm ³ Chamber |
| 0.30 | <i>Stegomyia (Aedes) aegypti</i> | 0.80 | 2.50 | 70 cm ³ Chamber |
| 0.30 | <i>Anopheles stephensi</i> | 1.00 | 2.50 | 70 cm ³ Chamber |
| 1.00 | <i>Stegomyia (Aedes) aegypti</i> | 0.30 | 1.14 | 2m ³ Peet-Grady chamber |
| 1.00 | <i>Culex quinquefasciatus</i> | 0.76 | 0.81 | 2m ³ Peet-Grady chamber |
| 1.00 | <i>Anopheles stephensi</i> | 0.90 | 1.68 | 2m ³ Peet-Grady chamber |
| 0.30 | <i>Culex pipiens pallens</i> | 0.12 | 8.80 | 70 cm ³ Chamber |
| 0.30 | <i>Anopheles stephensi</i> | 0.31 | 5.20 | 70 cm ³ Chamber |

| | | | | |
|------|----------------------------------|------|------|----------------------------|
| 0.30 | <i>Stegomyia (Aedes) aegypti</i> | 0.46 | 5.50 | 70 cm ³ Chamber |
| 0.15 | <i>Culex pipiens pallens</i> | 0.22 | 3.60 | 70 cm ³ Chamber |
| 0.15 | <i>Stegomyia (Aedes) aegypti</i> | 0.87 | 2.50 | 70 cm ³ Chamber |
| 0.15 | <i>Anopheles stephensi</i> | 0.88 | 2.70 | 70 cm ³ Chamber |
| 0.15 | <i>Culex pipiens pallens</i> | 0.38 | 2.80 | 70 cm ³ Chamber |
| 0.15 | <i>Stegomyia (Aedes) aegypti</i> | 0.59 | 1.80 | 70 cm ³ Chamber |
| 0.15 | <i>Anopheles stephensi</i> | 0.73 | 1.70 | 70 cm ³ Chamber |

These data is courtesy of Chadwick 1975, Yamaguchi *et al* 1981, Amalraj *et al* 1996 and Katsuda *et al* 2008 [42, 44, 45, 46]

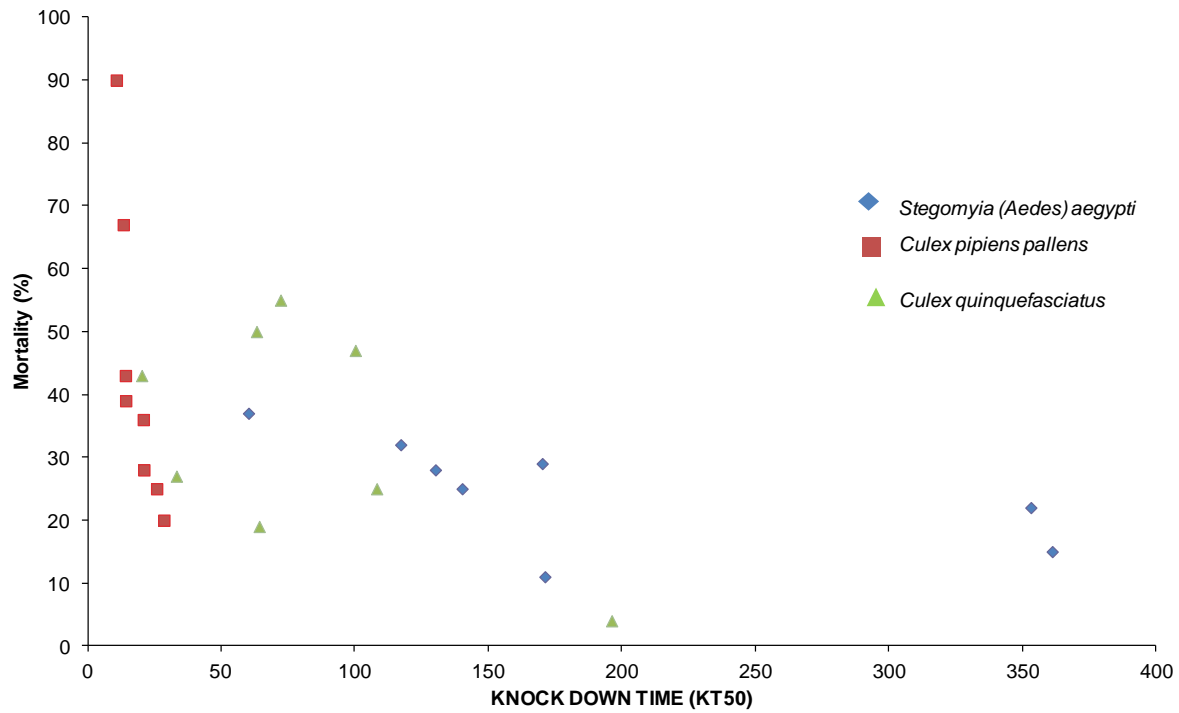


Figure 2.2: Mosquito species response of the relationship between KT50 and mortality. These data was extracted from Katsuda *et al* 2008 (Part 1)

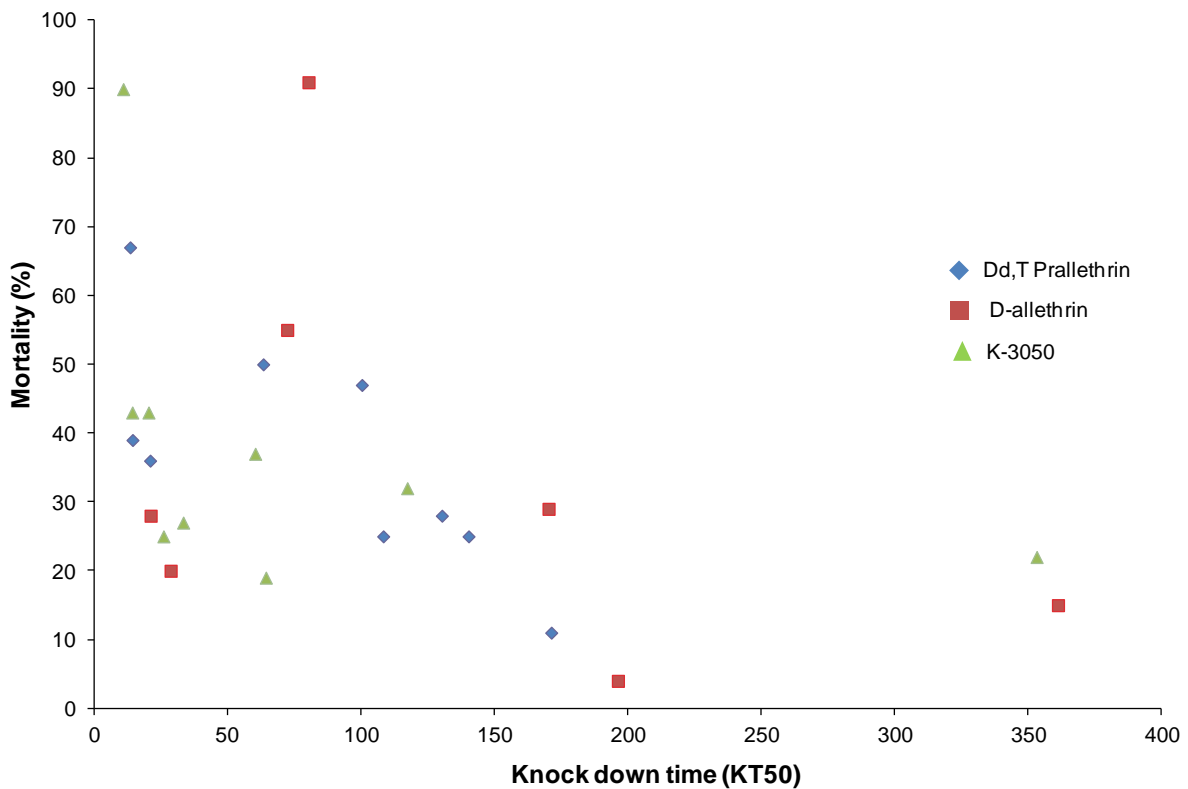


Figure2.3: The relationship between knock down time (KT50) and mortality of mosquitoes after exposure to mosquito coils impregnated with different doses of mosquito coils. These data was extracted from Katsuda *et al* 2008 (Part 1)

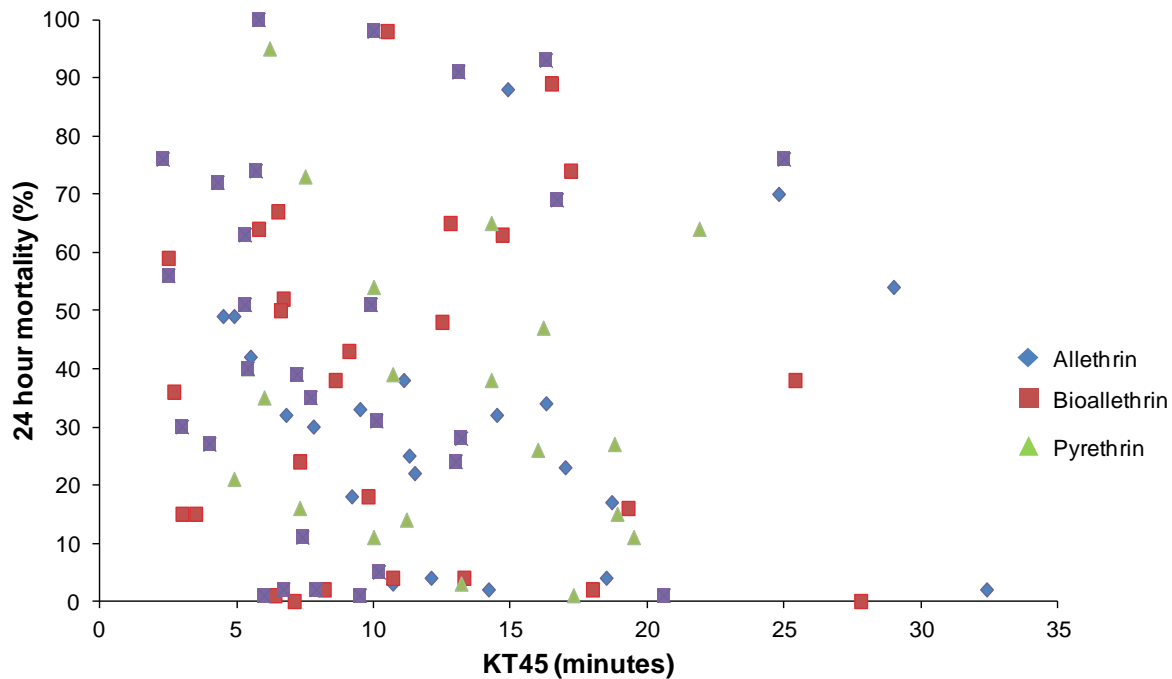


Figure 2.4: The relationship between knock down time (KT50) and 24 hour mortality in laboratory assays. These data was extracted from Chadwick *et al* 1975

Optimized metofluthrin emanators induce 100% KD of mosquitoes within 30 minutes of exposure followed by 100% mortality within 24 hours in the laboratory [28]. We did not find any studies that demonstrated correlation between dose and response of mosquitoes to emanators. However, Kawada *et al.* reported that caged mosquitoes placed immediately near metofluthrin-treated paper strips showed 100% KD within 30 minutes and 100% mortality 24-hours post-exposure, while mosquitoes placed 1.5m away from the strip had slower KD and 70% mortality and mosquitoes placed 5m away were unaffected [28]. This could be attributed to decreasing concentration of airborne active ingredients as one moved away from the source. It is noteworthy that these results may not be representative of natural

conditions because mosquitoes are confined within the cage thus are likely to take up a high concentration of the active ingredient compared to when they are free flying.

The intensity of KD and mortality of mosquitoes is largely dependent on release and degradation rates of active ingredients, initial loading dose on substrate and environmental conditions.

2.5. Harmonization in methodologies for testing spatial mosquito repellents

To characterize behavioural endpoints of mosquitoes exposed to chemical emanations of coils and emanators through rigorous independent and repeatable tests, it is essential to harmonize methodologies used.

2.5.1. Mosquito species

The mosquito species selected for bioefficacy studies is dependent on the objective and medical importance of a particular species in a given study area. The World Health Organization (WHO) recommends use of *Stegomyia (Aedes) aegypti* and *Culex quinquefasciatus* for testing household-insecticides [19]. Evaluations should be conducted on both susceptible and resistant strains of different mosquito species. Different mosquito genera, species, and population strains of the same species, vary in their susceptibility to insecticides due to specific selection pressures at site of origin and this can bias the intensity of outcome measures (Table 2.1.).

Consequently, we recommend that, when available, mosquito test populations should be acquired from disease endemic areas for which the chemical active ingredients are intended to be used.

2.5.2. Size of the laboratory test chambers or rooms

Field and laboratory studies are usually conducted in chambers, cylinders, rooms or huts of different sizes (Table 2.2.). Mosquitoes are knocked-down faster in cylinders or small chambers compared to large rooms (25m³) [42]. This is attributed to low aerial concentration of chemical actives in large ventilated rooms. Peet-Grady chambers [19] are good alternatives to air-tight cylinders. These chambers have improved ventilation provided by built-in fans and a larger volume (180cm by 180cm by 180cm) [19]. Tests carried out in Peet-Grady chambers and large rooms demonstrated that KD time was relatively shorter in the chambers than in the rooms [42]. Despite these limitations, cylinders, chambers and small rooms enable precise measurement of mosquito responses to various doses of chemical actives and generation of dose response curves. This might not be possible in field settings where external environmental factors such as wind speed and direction are likely to influence efficacy of the spatially acting actives. Cylinders or small chambers should be used primarily during initial screening for actives. Subsequent studies should then be conducted in more natural environments such as experimental huts and semi-field systems.

2.5.3. Environmental factors

The spatial activity of airborne insecticide is dependent on airflow (i.e., air exchange), wind speed, temperature and humidity within the treated space [47]. The greater the air current, the greater the insecticidal dispersion over a specified area followed by reduced insecticide concentration accompanied by dilution of chemical attractants from the human, leading to reduced host attack by mosquitoes [48]. A study carried out in Tanzania demonstrated reduced efficacy of emanators when used in houses with open eaves [25] compared to houses that did not have eaves in Vietnam [47]. It is necessary to consider the degree of ventilation of the test structure and average environmental conditions during peak disease transmission seasons within the test area where the spatial repellent will be used. High temperature increases evaporation rate of active ingredient [47] that may improve efficacy but can also lead to faster loss of actives followed by reduced efficacy over time. Therefore, it is necessary to determine the rate at which chemical actives are released from coils and emanators under different environmental conditions in order to determine how much repellent active ingredient will be required for efficacy over time.

2.5.4. Experimental design

Other factors affecting experimental outcomes include sample size, which may refer to the number of people used in the trial or number of mosquitoes used and the

number of replicates performed during evaluations. It is necessary to determine the number of mosquitoes required for a representative sample. This also applies to the number of human subjects required to account for differences in individual attractiveness to mosquitoes [49-51]. Wherever possible, a balanced Latin Square or William's Square design with rotation of volunteers and or treatments is desirable. We recommend analysis with generalised linear mixed models [52] which account for over-dispersed nature of repellent mosquito data when variance is greater than mean due to variability caused by the great variability among experimental days [53]. Few of the studies reviewed used appropriate study design or analyses. We propose that future studies should report means with standard errors or confidence intervals, or medians with the inter-quartile range in addition to test statistics. This information was not given by most of the studies reviewed, thus, we were unable to conduct a meta-analysis.

2.6. Conclusion

Spatial repellents is the general term used to describe delivery formats such as coils, mats and passive emanators which release vaporised chemical actives capable of affecting mosquito behaviour at a distance. Most vapour chemical actives also knock down, kill or inhibit feeding of mosquitoes. General use of this term causes confusion especially where oriented movement away from the chemical source is not demonstrated. For purposes of clarity we propose that spatial repellency should be used as a general term to refer to sum of mosquito behaviours induced by airborne

chemicals that cause mosquitoes to sit apart from a source of stimulation. Despite differences in evaluation methodologies, coils and emanators clearly reduce human-mosquito contact. They induce mortality, deterrence, repellency and reduce feeding of mosquitoes on humans. Mortality was the least observed effect where tests were conducted in experimental huts. This shows that these products do not kill mosquitoes in natural settings with free air movements and therefore may not affect overall mosquito densities or contribute to “community effect” as other toxic insecticides would.

Mosquito coils increased the proportion of mosquitoes exiting huts. It is not clear whether mosquitoes leave treated houses because they are unable to locate hosts for blood meals and therefore continue searching for other blood sources or whether they leave because they are irritated by chemicals in the smoke/vapour and are forced to escape. This needs more investigation.

Reduction in human-vector contact through feeding inhibition is likely to have an epidemiologically significant effect because of reduced risk of getting infectious mosquito bites. Any reduction in human-biting rate of mosquitoes is likely to lower vectorial competence of vectors and affect the lifetime fecundity of vectors which will in turn influence the basic reproductive rate of any parasites that they transmit. In addition to the measure of chemically induced feeding inhibition, it is necessary to conduct studies that quantify fecundity in order to see whether reduced blood feeding consequently reduces fertility of mosquitoes and leads to an overall reduction of mosquito population.

There is minimal data available on dose–response relationships, effective distance and residual efficacy of treated materials. However, the data reviewed here indicate that feeding inhibition, knockdown and mortality are positively influenced by high doses of active ingredient while deterrence does not change with change in dose. However, other studies indicate that deterrence resulting from DDT residues inside huts diminishes with time as the active ingredient degrades [3], indicating a dose dependent-response relationship. Unfortunately, there was no evidence from testing coils and emanators, hence there is need to conduct studies to ascertain this for different doses of coils and emanators under outdoor conditions.

It is hypothesized that since spatial repellents do not kill mosquitoes, there is increased risk of unprotected people being infected with pathogens transmitted by mosquitoes diverted from repellent users [54]. Therefore it is necessary to determine the distance at which non-users are at increased risk of receiving more mosquito bites for repellent-specific actives. On the other hand, non-users may in fact be protected due to airborne dispersion of volatilized chemicals. In addition, it is also worthwhile to understand whether feeding inhibition of mosquitoes can be prolonged over several hours or days through product optimization, as this is an epidemiologically significant endpoint for arthropod-borne diseases.

A meta-analysis could not be conducted as a result of the differences in evaluation methodologies as well as minimal statistical parameters reported by various studies. Hence, we strongly underline the need to reach consensus in spatial repellent testing methodologies and data reporting facilitated through the development of standardized assay guidelines. It is important to note that it is highly likely that

additional data on spatial repellents has been gathered but not made available to the scientific community. Publication bias due to industry-associated research may contribute to missing data sets, which if shared could greatly contribute to better characterization of spatial repellents. This information is vital for the development of standardized testing methodologies as well as target product profiles. Therefore scientists in industry are encouraged to share their data which will aid this process.

Spatial repellents have the potential to become an important component of vector control since outdoor biting vectors are gaining importance as malaria vectors [55]. In order to understand the dynamics of these products and their potential for vector control programs it is necessary to comprehensively characterize their mode of action (i.e., physiological pathways/receptors and behavioural modification involved in insect response) using standardized methodologies to facilitate the development of a target product profile (TPP) and testing of candidate products so that the required information on their efficacy in disease prevention can be more rapidly collected and policy makers better informed for maximum effective benefit in disease control.

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CHAPTER THREE

3 Screening Mosquito House Entry Points as a Potential Method for Integrated Control of Endophagic Filariasis, Arbovirus and Malaria Vectors

3.1. Abstract

Background: Partial mosquito-proofing of houses with screens and ceilings has the potential to reduce indoor densities of malaria mosquitoes. We wish to measure whether it will also reduce indoor densities of vectors of neglected tropical diseases.

Methodology: The main house entry points preferred by anopheline and culicine vectors were determined through controlled experiments using specially designed experimental huts and village houses in Lupiro village, southern Tanzania. The benefit of screening different entry points (eaves, windows and doors) using PVC-coated fibre glass netting material in terms of reduced indoor densities of mosquitoes was evaluated compared to the control.

Findings: 23,027 mosquitoes were caught with CDC light traps; 77.9% (17,929) were *Anopheles gambiae* sensu lato, of which 66.2% were *An. arabiensis* and 33.8% *An. gambiae* sensu stricto. The remainder comprised 0.2% (50) *An. funestus*, 10.2% (2359) *Culex* spp. and 11.6% (2664) *Mansonia* spp. Screening eaves reduced densities of *Anopheles gambiae* s. l. (Relative ratio (RR) = 0.91; 95% CI = 0.84, 0.98; P = 0.01);

Mansonia africana (RR = 0.43; 95% CI = 0.26, 0.76; P,0.001) and *Mansonia uniformis* (RR = 0.37; 95% CI = 0.25, 0.56; P,0.001) but not *Culex quinquefasciatus*, *Cx. univittatus* or *Cx. theileri*. Numbers of these species were reduced by screening windows and doors but this was not significant.

Significance: This study confirms that across Africa, screening eaves protects households against important mosquito vectors of filariasis, Rift Valley Fever and O’Nyong nyong as well as malaria. While full house screening is required to exclude *Culex* species mosquitoes, screening of eaves alone or fitting ceilings has considerable potential for integrated control of other vectors of filariasis, arbovirus and malaria.

3.2. Author Summary

Mosquito vectors that transmit filariasis and several arboviruses such as Rift Valley Fever, Chikungunya and O’Nyong nyong as well as malaria co-occur across tropical Africa. These diseases are co-endemic in most rural African countries where they are transmitted by the same mosquito vectors. The only control measure currently in widespread use is mass drug administration for filariasis. In this study, we used controlled experiments to evaluate the benefit of screening the main mosquito entry points into houses, namely, eaves, windows and doors. This study aims to illustrate the potential of screening specific house openings with the intention of preventing endophagic mosquitoes from entering houses and thus reducing contact between humans and vectors of neglected tropical diseases. This study confirms that while full

house screening is effective for reducing indoor densities of *Culex* spp. mosquitoes, screening of eaves alone has a great potential for integrated control of neglected tropical diseases and malaria.

3.3. Introduction

Houses are the main site for contact between humans and night biting mosquito vectors [1, 2]. The impact of improved housing on indoor malaria vector densities [3-7] and transmission [4] is well established. In Africa, the primary malaria vectors are nocturnal, endophilic and endophagic mosquitoes of the *Anopheles gambiae* species complex [8, 9]. These vectors prefer to enter houses via open eaves [2]. Therefore, houses with open eaves or those lacking ceilings have higher numbers of mosquitoes and a greater malaria burden compared to those with closed eaves or with ceilings [4-6, 10].

Regardless of evidence that improved housing provides protection from *Anopheles* malaria vectors, its potential to reduce indoor biting densities of other mosquito genera has received little attention, despite the fact that several of these species are known vectors of diseases which cause significant morbidity and mortality. These diseases include lymphatic filariasis, several arboviruses such as Chikungunya, O’Nyong nyong, Rift Valley Fever (RVF) and West Nile Virus (WNV) (Table 3.1).

An. gambiae sensu stricto and *An. arabiensis* are the most abundant malaria vectors in rural tropical African countries and are also the main vectors of filariasis [11] as well as O’Nyong nyong [12]. *Mansonia africana* and *Ma. uniformis* are vectors of RVF

and filariasis, although the latter predominantly transmits Brugian filariasis in Asia. Integrated control of filariasis and malaria is feasible [13, 14] due to their co-occurrence in rural areas, where they are often co-endemic and transmitted by the same vectors [15]. Though the main control measure against filariasis is chemotherapy, achieved through mass drug administration, a more holistic approach that integrates other proven interventions may be feasible in many endemic areas [16].

Culex quinquefasciatus is a vector of *Wuchereria bancrofti* causing lymphatic filariasis in Africa. It is the main vector in urban areas [17] but also contributes to rural transmission. *Cx quinquefasciatus* is also a vector of other arboviruses such as Chikungunya and West Nile Virus (Table 3.1.). Several other *Culex* species transmit other arboviruses in East Africa; these are shown in Table 3.1.

Crucially, culicines are also the major cause of nuisance biting in rural and especially urban areas [18]. Several studies have shown that the community is sensitive to changes in biting nuisance related to changes in mosquito densities. Uptake of several control measures such as use of house screens [19] and mosquito coils [20] is dependent upon the desire to prevent mosquito bites in addition to preventing diseases. Similarly, use of insecticide treated nets (ITNs) is motivated by the desire to prevent nuisance bites [21, 22], as shown by reduction in the use of ITNs when mosquito densities are lower due to seasonal decline [23, 24] even when mosquito numbers are sufficient for disease transmission to continue.

Unfortunately, efficacy of insecticide based interventions declines when resistance develops, as has already been seen in Tanzania [25, 26]. If people continue to be bitten

by nuisance mosquitoes due to development of insecticide resistance, it undermines public acceptance of ITNs as an intervention [27, 28]. Therefore, there is need to develop supplementary tools for control of nuisance mosquitoes. Reduction in nuisance mosquitoes will increase users' confidence in the available mosquito control measures and therefore also encourage use of other measures.

The aim of the study was to evaluate preferential points of entry of different mosquito species into houses. This was determined by indoor densities of different species of mosquitoes when a specific entry point was screened, precisely, eaves, windows and doors compared to an unscreened control. Our overall goal was to evaluate the optimal method needed for house screening in order to provide integrated control of filariasis, arboviruses and malaria vectors.

Table 3.1: Mosquitoes naturally infected with arboviruses or *Bancroftian filariasis* in southern and eastern Africa.

| Species | Disease carried | Country | References |
|---------------------------------------|------------------------|----------------------------------|--------------|
| <i>Anopheles gambiae s.l.</i> | O’Nyong nyong | Uganda, Kenya, Mozambique | [12] |
| | Bancroftian filariasis | Tanzania | [11] |
| <i>Anopheles funestus</i> | O’Nyong nyong | Uganda, Kenya, Mozambique | [12] |
| | Bancroftian filariasis | Tanzania | [11] |
| <i>Mansonia africana</i> | Rift Valley Fever | Kenya | [29] |
| | | Uganda | [30, 31] |
| | Chikungunya | Uganda | [12] |
| <i>Culex pipiens quinquefasciatus</i> | West Nile Virus | Madagascar | [32] |
| | Chikungunya | Tanzania | [33] |
| | Bancroftian filariasis | Tanzania | [11] |
| <i>Culex univittatus complex</i> | Sindbis Virus | South Africa | [34, 35] |
| | West Nile Virus | South Africa | [34, 36] |
| <i>Culex theileri</i> | | Madagascar | [32] |
| | West Nile Virus | Kenya | [32, 37] |
| | | South Africa | [12, 32] |
| | Rift Valley Fever | South Africa | [38, 39] |
| <i>Culex rubinotus</i> | Witswatersrand | Uganda, Mozambique, South Africa | [12, 30, 40] |

3.4. Methods

3.4.1. Study site

The experimental hut study was carried out at Lupiro village (8.01 °S and 36.63 °E) located in Ulanga district, in the south eastern part of Tanzania. The village lies 300 meters above sea level on the flood plain of Kilombero River, approximately 26 km south of Ifakara town. The climate is hot and humid, experiencing annual rainfall ranging between 1200–1800 mm and annual mean temperature between 20–32 °C. This climate and the clearance of a perennial swamp for rice farming create ideal conditions for perennially abundant populations of both *An. gambiae* s. s. and *An. arabiensis* and many species of culicine mosquitoes [41]. Malaria transmission intensity in this village is exceptionally high, averaging between 474 and 851 infectious bites per person per year, despite mosquito net coverage which consistently exceeds 75% [42]. In addition, there have been several cases of RVF and filariasis (E. Mossdorf pers comm).

3.4.2. Local houses

In Ulanga and Kilombero DSS (Demographic Surveillance System) areas, most of the local houses have mud walls (56%), while the remainder is made of baked mud bricks. The roofs are mostly thatched (70%) or made of corrugated iron. The houses chosen for these experiments therefore had mud walls and thatched roofs with open eaves and one or two windows (Figure 3.1.). Cooking was mainly done outside of the hut and each of the local houses selected had two or three people living in them.



Figure 3.1. A local house. The local houses are made of mud walls and thatched roofs. They have one door and two windows and open eaves (open spaces between the roof and the wall).

3.4.3. Experimental huts

Several prototypes of new designs of experimental huts (Unpublished Moore et al) were built in Lupiro (Figure 3.2.) with the intention of representing, as closely as possible, the key structural features of local housing in southern Tanzania (i.e. brick or mud huts with corrugated iron or thatched roofing). These huts were designed in kit form for ease of portability, with a galvanized piping framework so that the entire hut could be flat packed. The roof is corrugated iron covered with grass thatch on the top, to simulate the temperature of local houses with thatched roofing. The outer walls are constructed from wooden planks or canvas. The inner walls are removable panels coated with mud, to simulate local mud walls. Two huts were constructed to mimic average local huts in the village. These were 6.5 m long, 3.5 m wide and 2 m high, (the size of these huts was determined by measuring 100 houses in Lupiro and calculating the average dimensions). The remaining two were smaller, at 3 m long, 3.5 m wide and 2 m high. The height of each structure measured 2.5 m at the roof apex. Each experimental hut had one door and two window openings as this was the median number seen in local houses.



Figure 3.2. A wooden experimental hut. The experimental huts were designed to represent local housing in southern Tanzania. An experimental hut had a corrugated roof and covered with grass thatch on the top, to simulate the temperature of local houses with thatched roofing. The outer walls were constructed from wooden planks or canvas. The inner walls were made of removable panels coated with mud. They had one door and two functional windows with open eaves (open spaces between the roof and the walls).

3.4.4. Experimental design

Two blocks of four huts were used for these experiments: one block of four local houses and one block of four experimental huts. The selected houses were located nearest to the experimental huts and were selected to be approximately 50 m apart from each other. Two male volunteers slept in each experimental hut. The volunteers were not rotated between huts but remained in the same hut for the duration of the study. The bias created by variation in human attractiveness to mosquitoes and spatial variation between huts were therefore combined and treated as a single source of bias in the statistical analysis. For each of the two blocks of four houses, the following sequence of experimental treatments was completed. In each block, four repetitions of four experimental treatment arrangements were completed between 4th December and 19th December 2007. This is the peak of short rains and therefore there is wide spread flooding leading to high densities of mosquitoes of all genera. Each repetition included three nights during which three of the four houses had the same one of the three potential entry points screened while the remaining fourth house was completely unscreened. On the first night of each repetition, all the four huts remained completely unscreened. For the subsequent three nights of each repetition, all the three treatments were changed each night from screening the eaves to windows and then doors, in that order. For each night, a different hut was chosen within each block to have no entry point screened, so that at the end of the four repetitions, all four huts had acted as these contemporaneous controls. The treatments were rotated across all the huts systematically. Rotation of treatments reduced the bias of mosquito collections

between the huts.

3.4.5. Screening entry points

PVC-coated fibre glass netting material (Elastic Manufacturing, Tanzania) was used to screen specific entry points each particular night. The netting was cut to fit each of the entry points (doors, windows and eaves). In the experimental huts, the size of the windows, eaves and doors was uniform for all the huts. Screens were fitted on the experimental huts by hook and loop fasteners. In the local houses, the screens were nailed onto the wall (mud wall). The nails could be removed easily each morning at the end of the experiments. Due to uneven wall surfaces of the local huts, small gaps were found between the netting and the wall. These gaps were blocked with cotton wool to create a complete barrier.

3.4.6. Mosquito collection

CDC light trap is an appropriate tool for sampling mosquito vectors that would otherwise bite humans, thus being comparable to human landing catches [43-46]. A CDC miniature light trap (model 512) was positioned approximately 1 m above the ground. It was placed next to the bed (at the foot end) occupied by an adult male volunteer, under an untreated bed net [45]. Volunteers operated light traps from 19:00 to 07:00 hrs each night.

Although no attempt was made to control times at which occupants slept, this period

typically approximated 19:00 hrs to 07:00 hrs. Traps were collected from each house every morning at 07.00. Collection bags were then placed in a plastic bucket, and mosquitoes were killed using cotton wool treated with chloroform.

3.4.7. Mosquito identification

The mosquitoes were morphologically identified to genus level each morning in the field while they were still fresh. Mosquitoes were stored in small centrifuge tubes which contained tissue paper with silica gel beneath, then transported to the laboratory where they were stored at 20°C, until further identification. Further identification was done to species level using polymerase chain reaction (PCR) for *An. gambiae* s. l. [47]. Mosquitoes allocated for PCR were sampled randomly from *An. gambiae* s. l., mosquitoes collected from different trap nights by placing labelled tubes in a box and picking them at random. Morphological identification of culicines was done using a key [47].

3.4.8. Ethics

Volunteers were recruited only if they agreed to participate in the study and signed a written informed consent form. To minimize risk of infection of mosquito borne diseases, participants were provided with untreated nets. In addition, they were offered free malaria screening and treatment. Ethical approval was granted by Ifakara Health Institute (IHI) (IHRDC/IRB/No. A- 014-2007, IHRDC/IRB/No.A-019-2007) and the National Institute of Medical Research (NIMR/HQ/R.8a/Vol. W710). The Centre for Disease Control (CDC) ethical review deemed the work non- human subjects research.

3.4.9. Statistical analysis

Generalized estimating equations were used with SPSS 15 to estimate the effect of screening specific entry points, which was treated as a categorical independent variable, on indoor mosquito densities relative to unscreened controls. House number was fitted as a subject effect and day as the within-subject variable, with an exchangeable working correlation matrix, to account for spatial and temporal heterogeneity in the dependent variable, namely number of mosquitoes of a given mosquito taxon caught in each house on each night. Note that, each species was analyzed separately using generalised estimating equation models (GEE). *An. gambiae* s. l. mosquito catch had a normal distribution and was fitted to an identity link. All the other species were negatively skewed and were therefore fitted with a negative binomial and a log link function. The model was used to derive the relative rates and their 95% confidence intervals.

Binary logistic regression was used to test the strength of the influence of different treatments on the proportion of *An. arabiensis* and *An. gambiae* s. s caught that were identified to sibling species by PCR. The independent variables fitted in the model were treatment and house number. The outcome variable was binomial; *An. arabiensis* and *An. gambiae* s. s were coded as 1 and 0 respectively and the effect of treatment on the odds ratio of finding *An. arabiensis* relative to *An. gambiae* s. s. was calculated.

3.5. Results

3.5.1. Mosquito collections

During the cumulative 16 nights of sampling, with the CDC light traps, 77.9% (17,929) of the total catch were *Anopheles gambiae* s. l. This species complex comprised 66.2% (738) *An. arabiensis* and 33.8% (n = 377) *An. gambiae* s. s (n = 1115 successful PCR amplifications). There were only 0.2% (n = 50) *An. funestus* species complex caught in the entire study. One tenth (10.2%, n = 2359) of all mosquitoes collected were various *Culex* spp. Three quarters (76.9%) of *Culex* spp. were identified as *Cx. pipiens* complex of which four fifths (80.3%, n = 875) were *Cx. pipiens quinquefasciatus*, while the remainder (19.7%, n = 214) were *Cx. pipiens pipiens*. Other culicines included *Cx. univittatus* and *Cx. theileri* (20.0% of the total *Culex* spp). Just over one tenth (11.6%) of all mosquitoes collected were *Mansonia* spp., of which more than half (58.3% n = 1038) were *Ma. uniformis* and the remaining 41.6% (n = 742) were *Ma. africana*. Other species of culicines caught in smaller numbers were, *Cx. horridis* (n = 7), *Cx. andersanius* (n = 11), *Cx. acrostichalis* (n = 43), *Cx. rubinotus* (n = 30), *Cx. sitiens* (n = 5), *Cx. simpsoni* (n = 18), and *Cx. aureus* (n = 69).

3.5.2. Effect of screening different entry points on indoor densities

A summary of the median indoor density species collections when each entry point was screened is presented in Table 3.2 and a statistical estimate of the impact of

screening is presented in Table 3.3.

An. gambiae s. l. mosquitoes were less likely to be found in houses with screened eaves (Table 3.3.). Binary logistic regression revealed that both treatment (screening of various entry points) and house did not affect the proportion of *An. gambiae* s. s. versus that of *An. arabiensis* mosquitoes, (Treatment, Odds Ratio [95% confidence interval] = 1.06 [0.94, 1.20]; Wald Chi square = 0.87; P = 0.35), indicating that the effect of treatment on the two sibling species was similar. Screening eaves also reduced both *Ma. africana* and *Ma. uniformis* mosquito densities by almost half (Table 3.3.). Screening windows and the door reduced indoor densities of *Cx. quinquefasciatus*, *Cx. theileri* and *Cx. univittatus* mosquito densities by a quarter or more although this was not significant (Table 3.3.). The relative densities of *Cx. univittatus* and *Cx. theileri* mosquitoes were increased when eaves were screened respectively (Table 3.3.).

Table 3.2: Median indoor densities of different mosquito species caught in experimental huts and local houses when different entry points were screened.

| Screened entry points | | None ^a | | Eaves | | Windows | | Doors | |
|----------------------------------------|------|-------------------|------|--------------|------|--------------|------|--------------|--|
| Mosquito species | n | Median [IQR] | n | Median [IQR] | n | Median [IQR] | n | Median [IQR] | |
| <i>An. gambiae sensu lato</i> | 8341 | 80.0[4,630] | 2708 | 59.0[9,415] | 2946 | 80.0[15,370] | 3934 | 96.0[17,700] | |
| <i>Ma. africana</i> | 336 | 3.0[0,31] | 144 | 0.0[0,12] | 138 | 3.0[0,24] | 144 | 1.0[0,36] | |
| <i>Ma. uniformis</i> | 584 | 3.5[0,66] | 93 | 1.5[0,21] | 198 | 6.0[0,36] | 163 | 1.5[0,37] | |
| <i>Cx. quinquefasciatus sensu lato</i> | 544 | 2.0[0,79] | 206 | 2.0[0,40] | 171 | 2.0[0,46] | 168 | 0.0[0,50] | |
| <i>Cx. theileri</i> | 27 | 0.0[0,5] | 28 | 0.0[0,8] | 4 | 0.0[0,2] | 9 | 0.0[0,5] | |
| <i>Cx. univittatus</i> | 60 | 0.0[0,11] | 49 | 0.5[0,10] | 19 | 0.0[0,5] | 16 | 0.0[0,4] | |

n = number of mosquitoes. ^a =Reference group (No entry point was screened). IQR = Interquartile range. A total of 127 experimental hut nights were conducted. The number of experimental nights conducted for screened entry points are: None – 56, Eaves – 24, Windows – 23 and Doors – 24. A CDC Light trap on an eave night was attacked by ants, thus no data was recorded for that particular hut night.

Table 3.3: Impact of screening various entry points upon indoor densities of different mosquito species caught with reference to indoor densities when no entry point was screened.

| Screened entry points | None ^a | Eaves | | Windows | | Doors | |
|----------------------------------------|-------------------|------------------------|------------------|-----------------|-------|-----------------|-------|
| Mosquito species | | RR [95%CI] | P | RR [95%CI] | P | RR [95%CI] | P |
| <i>An. gambiae sensu lato</i> | 1 | 0.91[0.84,0.98] | 0.010 | 0.98[0.94,1.02] | 0.340 | 1.03[0.97,1.09] | 0.310 |
| <i>Ma. africana</i> | 1 | 0.43[0.26,0.76] | <0.001 | 0.91[0.58,1.44] | 0.700 | 1.03[0.63,1.70] | 0.900 |
| <i>Ma. uniformis</i> | 1 | 0.37[0.25,0.56] | <0.001 | 0.85[0.54,1.33] | 0.470 | 0.65[0.38,1.13] | 0.130 |
| <i>Cx. quinquefasciatus sensu lato</i> | 1 | 0.91[0.50,1.65] | 0.740 | 0.77[0.42,1.39] | 0.380 | 0.72[0.36,1.45] | 0.360 |
| <i>Cx. theileri</i> | 1 | 2.42[1.13,5.18] | 0.020 | 0.36[0.11,1.22] | 0.100 | 0.78[0.25,2.46] | 0.670 |
| <i>Cx. univittatus</i> | 1 | 1.91[1.05,3.47] | 0.040 | 0.77[0.37,1.61] | 0.490 | 0.62[0.31,1.25] | 0.180 |

The relative rates (RR), model estimated 95% confidence intervals (CI) and probability of equivalence (P) were all estimated by Generalized estimating equations as described in the Methods section.^a = Reference group (No entry point was screened).

3.6. Discussion

More than three quarters of the mosquitoes caught during the study were *An. gambiae s. l.* a major vector of both lymphatic filariasis as well as malaria in this area and across most of Africa [11]. *An. funestus* complex mosquitoes caught in this study were not identified to species level. However, other studies from Tanzania have shown that this species complex shows distinct behavioural differences. *An. funestus s. s.* mosquitoes are mainly endophagic while others like *An. rivulorum* are mainly exophagic [48]. Therefore, since mosquitoes were collected indoors we assume that most of the mosquitoes caught were *An. funestus s. s.*

Culicine mosquitoes collected in this study contribute to the transmission of filariasis and arboviruses (Table 3.1). *Cx. quinquefasciatus* was the most abundant *Culex* spp. caught. Significant numbers of *Cx. univittatus* and *Cx. theileri* mosquitoes were also caught. *Ma. africana* has been incriminated as a vector of RVF [29-31], and was present in high densities during an outbreak of RVF among humans at the field site (E. Mosssdorf pers comm).

Most of the mosquitoes caught were unfed, and therefore considered to be in the act of host seeking [43, 44]. Studies carried out previously in the same experimental huts (unpublished data) indicated that there were very low densities of indoor resting mosquitoes. Only 0.35% of the mosquitoes caught in that particular study were caught resting. Therefore it may be assumed that indoor resting mosquitoes were present in insufficient numbers to bias the outcome of the screening experiments.

Consistent with previous reports [5-7], *Anopheles gambiae s. s.* and *An. arabiensis*

mosquitoes were noted to prefer eaves as the main entry point, demonstrated by reduced indoor densities when this particular entry point was screened. Both *Ma. africana* and *Ma. uniformis* also preferred entry via eaves as exhibited by reduced indoor densities when eaves were screened. This data indicates that some mosquito borne diseases could be prevented by blocking eaves [2].

A study carried out in the Gambia showed a reduction in culicine indoor densities in houses with closed eaves but in association with horses tethered outside and with increased room height [49]. Indoor *Cx. pipiens* s. l. densities were reduced by 38% when eaves were closed [49]. On the contrary, a second study recently carried out in The Gambia measured the impact of closing eaves in addition to screening the doors in houses with no windows. The same study indicated that there was no additional reduction in culicine mosquito densities when eaves were blocked [50]. In the present study, we have shown that *Cx. quinquefasciatus*, *Cx. univittatus* and *Cx. theileri* mainly prefer windows and doors as their main point of entry. It is also important to note that when eaves were screened and windows and doors were left open, indoor densities of *Cx. univittatus* and *Cx. theileri* mosquitoes were increased in comparison to when all the three entry points were left unscreened. This indicated the importance of screening all the three entry points to achieve control of *Culex* spp. mosquitoes.

Effectiveness of house proofing on mosquito vectors depends on the interaction between their feeding behaviour and human behaviour especially when and where people eat and sleep [51-53]. House screening will only reduce exposure to endophagic mosquito vectors. Several anophelines in Africa are endophagic; therefore, house screening would be highly effective. Since most *Culex* spp. mosquitoes are commonly thought to be predominantly

exophagic, then it raises concerns of whether house screening would be effective against them. However, varying levels of both endophagy and exophagy observed in different species; differ from one region to another. In East and West Africa *Cx quinquefasciatus* is more endophagic [54]. *Cx. univittatus* and *Cx theileri* exhibit both exophagy and endophagy in some areas [55-57].

Our findings suggest that screening eaves reduces indoor densities of *An. gambiae* s. l. as well as *Mansonia* spp. both of which are vectors of several neglected tropical diseases in rural areas of Africa and some parts of Asia. Blocking eaves and full house screening, as a control tool against mosquito vectors may reduce nuisance mosquitoes and thus encourage uptake of control interventions that rely on acceptance, participation and even investment by the community.

Screening of eaves and/or installation of ceilings may prove to be practical and affordable where existing house designs prove amenable to such modifications. While most of the African population does not live in houses as uniform as our experimental huts, it is encouraging that mosquito proofing of houses by screening the eaves or installing ceilings has proven equally effective for anophelines and some culicines in rural settings in both East and West Africa. Blocking the eaves of the mud-walled, thatch-roofed village houses included in this Tanzanian study yielded results which are remarkably consistent with those observed when netting ceilings and screened eaves were installed into typical houses in The Gambia despite the wide geographical separation between them [5].

Recent evidence from urban Dar es Salaam [19] suggests that communities perceive closed ceilings and window screening as successful means to prevent house entry by mosquitoes.

They demonstrate high levels of acceptance, uptake and even investment, despite the fact that this intervention has never been specifically promoted on this basis. We suggest that the true full potential of protecting houses against house entry by culicine and anopheline mosquitoes, could be better achieved through insecticide treated screening material for targeted killing by placing them on either eaves, windows and doors.

3.7. References

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CHAPTER FOUR

4 An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission

4.1. Abstract

Background

Current malaria vector control programmes rely on insecticides with rapid contact toxicity. However, spatial repellents can also be applied to reduce man-vector contact, which might ultimately impact malaria transmission. The aim of this study was to quantify effects of airborne pyrethroids from coils and DDT used as an indoor residual spray (IRS) on entomological parameters that influence malaria transmission.

Methods

The effect of Transfluthrin and Metofluthrin coils compared to DDT on house entry, exit and indoor feeding behaviour of *Anopheles gambiae sensu lato* were measured in experimental huts in the field and in the semi-field. Outcomes were deterrence - reduction in house entry of mosquitoes; irritancy or excito-repellency - induced premature exit of mosquitoes; blood feeding inhibition and effect on mosquito fecundity.

Results

Transfluthrin coils, Metofluthrin coils and DDT reduced human vector contact through

deterrence by 38%, 30% and 8%, respectively and induced half of the mosquitoes to leave huts before feeding (56%, 55% and 48%, respectively). Almost all mosquitoes inside huts with Metofluthrin and Transfluthrin coils and more than three quarters of mosquitoes in the DDT hut did not feed, almost none laid eggs and 67%, 72% and 70% of all mosquitoes collected from Transfluthrin, Metofluthrin and DDT huts, respectively had died after 24 hours.

Conclusion

This study highlights that airborne pyrethroids and DDT affect a range of anopheline mosquito behaviours that are important parameters in malaria transmission, namely deterrence, irritancy/excito-repellency and blood-feeding inhibition. These effects are in addition to significant toxicity and reduced mosquito fecundity that affect mosquito densities and, therefore, provide community protection against diseases for both users and non-users. Airborne insecticides and freshly applied DDT had similar effects on deterrence, irritancy and feeding inhibition. Therefore, it is suggested that airborne pyrethroids, if delivered in suitable formats, may complement existing mainstream vector control tools.

4.2. Background

Currently, malaria vector control is focused on two interventions: Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Nets (LLINs) that have successfully reduced malaria transmission throughout sub-Saharan Africa [1]. In public health vector control programmes, efficacy of insecticidal tools (LLINs and IRS) is measured by the epidemiological endpoints: malaria mortality and morbidity, related to reduced intensity of transmission in the mosquito vector population [2]. Mathematical models have been used to

explain dynamics of malaria transmission through entomological, immunological and parasitological parameters that influence malaria transmission [3] expressed as the basic reproductive rate (R_0). The basic reproductive rate refers to the number of secondary disease infected persons arising from a single infected person in a completely susceptible population [2]. Therefore, the object of any control intervention is to reduce R_0 to less than 1.

Garrett-Jones [4] described the relationship between entomological parameters that influence malaria transmission, termed the vectorial capacity of a mosquito population. . The parameters of the entomological equation include mosquito abundance (m), mosquito daily survival (p) (the vector must live long enough for parasites to develop to the infective stage inside the mosquito) and frequency of contact between mosquitoes and humans through the man biting rate (ma). Vectorial capacity is defined as the expected number of new human malaria infections disseminated per human per day, by a mosquito population from a single case, presuming that all vector females feeding on the case become infective [2].

4.2.1. Vectorial capacity equation

The vectorial capacity equation as described by Garrett-Jones is as follows: $C = ma^2p^n / \log_e p$. C = vectorial capacity, ma = density of mosquitoes per person per night, a^2 = average frequency of biting on humans per day (a is squared because a mosquito must bite twice; 1st to receive parasites and 2nd to transmit them), p = the probability of daily survival of the mosquito and n = the duration of sporogony i.e the time required for the parasites to develop

in the mosquito (extrinsic period).

According to the vectorial capacity equation, changes to different aspects of the life cycle of mosquitoes will have differential impacts on malaria transmission [5]. For instance, a reduction in mosquito density (m) leads to an equal reduction in vectorial capacity because of their linear relationship, while a reduction in biting rate (ma) leads to a two-fold reduction in transmission due to the quadratic relationship (arising from the fact that mosquitoes need to feed twice to transmit malaria: once to become infected and once to infect) [5]. Importantly, interventions that affect the survival rate (p) of mosquitoes have the greatest impact on transmission due to their exponential relationship [5, 6]. Therefore, it becomes obvious why LLINs are such a successful vector control tool: they reduce man-vector contact (ma) because they create a barrier between mosquitoes and humans, reduce mosquito average daily survival (p) through their insecticidal mode of action and therefore also affect mosquito density (m).

Although the primary entomological modes of action (ENMoA) of insecticides used for LLINs and IRS are rapid knockdown and mortality, studies have shown other effects of insecticides that include 1) deterrence: when mosquitoes are prevented from entering human dwellings treated with insecticides [7, 8]; 2) irritancy: when mosquitoes contact insecticide surfaces inside houses and leave early [7]; 3) excito-repellency; when mosquitoes contact airborne insecticides and leave the house and 4) feeding inhibition; when mosquitoes are prevented from biting and getting blood meals [7]. The ENMoA of insecticides affect various aspects of the mosquito life cycle and this largely influence the success of any intervention. Despite emphasis placed on the importance of toxic insecticides, studies show that some highly effective insecticides, such as DDT (dichlorodiphenyltrichloroethane), are

primarily spatial repellents and feeding inhibitors [9] while toxicity is a lesser, but still important feature [9, 10]. In fact, the success of DDT is attributed to its deterrence and irritancy, and only to a lesser extent to its mortality [10, 11].

Mosquito coils, vaporizer mats and emanators also induce repellency, irritancy, feeding inhibition and toxicity [12, 13]. The impact of coils and emanators on vector borne diseases has been proven. These tools act over a distance by evaporating insecticides into a given space, hence are known as spatial repellents. This mode of action has parallels with the deterrent, feeding inhibition and excito-repellent modes of action of DDT. For this reason, it is worthwhile to compare their effects on entomological components that pertain to vectorial capacity. It is hypothesized that insecticides that have more than one mode of action affect different parameters of the vectorial capacity (m, a, ma, p,) and are likely to bring forth greater changes in transmission than anticipated if only toxicity is considered.

The purpose of this study was to quantify the effect of airborne pyrethroids released by mosquito coils on mosquito behaviour. Emphasis was placed on outcome measures that influence entomological parameters of malaria transmission (Table 4.1) and to compare the mode of action of Transfluthrin and Metofluthrin coils against DDT, representing a gold standard insecticide with known impact on malaria transmission [11].

Table 4.1: Entomological parameters of the vectorial capacity targeted by effects of airborne insecticides on mosquito behaviour

| Effect of airborne insecticides | Parameter of the vectorial capacity | System of study |
|---------------------------------------------------------------------|--------------------------------------------|------------------------|
| Deterrence | ma | Field |
| Excito-repellency and irritancy | ma | Semi-field |
| Toxicity | m, p | Field and semi-field |
| Reduced fecundity (ability of mosquitoes to lay eggs) | m | Semi-field |
| Feeding inhibition (mosquitoes prevented from blood feeding) | ma | Semi-field |

4.3. Methods

Studies were conducted in experimental huts in the field with wild *Anopheles arabiensis* mosquitoes and in a semi-field system [14] with laboratory reared *Anopheles gambiae sensu stricto* (s.s.) as a standard test organism for repellents [15]. The overall objective was to determine the effect of DDT, Metofluthrin and Transfluthrin coils on parameters of vectorial capacity using experimental huts.

4.3.1. Outcomes measured in the field

Deterrence

Deterrence refers to reduced house entry of mosquitoes resulting to reduced indoor densities. It was determined by comparing the total number of mosquitoes in huts with insecticides to control huts. The total number of mosquitoes inside huts included: live and dead mosquitoes in exit traps, dead mosquitoes found on the floor as well as mosquitoes found resting inside the hut.

Toxicity

Toxicity of coils and DDT was determined by comparing the proportion of dead versus live mosquitoes in insecticide huts to the control huts. Mosquitoes collected from huts were kept for 24 hours in an insectary after which mortality was recorded.

4.3.2. Outcomes measured in the semi-field

Contact irritancy and excito-repellency

Contact irritancy and excito-repellency refer to the rate at which mosquitoes exit huts after physical contact with insecticide treated surfaces or airborne insecticides, respectively. The exit rate is the proportion of female mosquitoes found in the exit traps at the top of every hour compared with the total number found inside huts (resting or dead on the floor) relative to the control hut. The increased or premature exit of mosquitoes is the estimated irritancy or excito-repellency [16] of insecticides used in the house.

Toxicity

The number of dead versus live mosquitoes out of those recaptured was compared between huts. Mortality was recorded after 24 hours. The difference in mortality between a control hut (natural mortality) and a treated hut allows assessment of the insecticide-induced mortality [16].

Blood feeding inhibition

Feeding inhibition was determined by comparing the number of blood fed versus unfed mosquitoes of total mosquitoes recaptured from huts.

Reduced fecundity of mosquitoes

Fecundity was determined by comparing the proportion of blood fed mosquitoes that laid eggs after exposure to different treatments compared to the control. In addition, the total number of eggs laid by each mosquito was determined.

4.3.3. Experiment 1: field

Study area

The study was conducted in Lupiro village in the Kilombero valley in the South East of Tanzania. Annual rainfall ranges between 1200 and 1800 mm with two rainy seasons per year: November to December and January to April. Annual mean temperature ranges between 20-32°C. Communities in Lupiro practice irrigated rice farming that provides suitable mosquito breeding conditions. *Anopheles arabiensis* is the dominant species (>95% of the malaria vector population) with the remainder comprising *Anopheles funestus sensu lato* (*s.l.*) mosquitoes. There is a high density of culicines comprised of *Culex* and *Mansonia* species [17]. A study conducted at the same time and site indicated 100% susceptibility of *An. arabiensis* mosquitoes to World Health Organization recommended doses of DDT and between 95.8% and 90.2% for Permethrin, Lambda cyhalothrin and Deltamethrin [18].

Treatments

Mosquito coils were used at a standard dose recommended and approved by the World Health Organization for Pesticides (WHOPES). They included Transfluthrin (0.03%) and Metofluthrin coils (0.00625%). Seventy-five percent pure DDT wettable powder (AVIMA, South Africa) was applied to woven palm leaf mats using Hudson sprayers at 2 g/m² concentration of the active ingredient. DDT was sprayed on mats that could be rotated between huts during experiments. Rotation of treatments between huts is a crucial part of experimental hut study design because it minimizes the spatial bias between huts that often

affects relative mosquito density and behaviour.

Palm woven mats were measured and cut out to fit the entire surface of the inside wall of an experimental hut. The reverse side of the mats was covered with plastic sheets (Figure 1) to prevent contamination of experimental hut surfaces with DDT during rotation of mats between huts. Two sets of mats were prepared, the control was sprayed with water and the other set was sprayed with DDT at a dose of 2 g/m^2 as recommended by WHOPES [16] using a separate Hudson sprayer for each treatment. The quantity of DDT required to cover walls of one hut was determined by measuring the surface area of walls. The amount of DDT required in g/m^2 was calculated and weighed. The volume of water required for mixing DDT was determined by pouring a known amount of water in a Hudson sprayer. The sprayers were calibrated to 55 psi and control mats were sprayed with water. The volume of water used in the control was measured and an equal volume of water was used for mixing DDT in a plastic bucket. Spraying was conducted in a disposable tent located 50 meters from experimental huts (Figure 4.1). The mats were air dried for 15 minutes then fixed to respective walls using removable staples so that they could be detached easily during rotation (Figure 4.1).



Figure 4.1. Spraying palm woven mats with DDT. Palm woven mats previously cut out to fit on the walls of experimental huts were sprayed with 2 g/m^2 DDT. Spraying was conducted in a temporary structure that was later burnt. Spraying the mats instead of the walls ensured that mats could be moved easily from one hut to another without contaminating the walls. This allowed rotation of treatments between huts.

Experimental huts

Studies were conducted in Ifakara experimental huts [19] (Figure 3.2). Initially, information about the size, design of the houses and the materials required for constructing the roofs and walls was collected through a house hold survey conducted in Kilombero valley. The local houses (Figure 3.1) in this region are constructed with corrugated iron sheets or thatched roofing and walls are constructed with bricks or mud. This information was used in the construction of experimental huts to ensure a good representation of local houses in Kilombero valley. The experimental huts measure 6.5 m long, 3.5 m wide and 2.5 m high at the roof apex. They are made of galvanized pipe framework, the roof is made of corrugated iron sheets and the inner walls are made of removable mud panels while outer walls are covered with canvas. The outer roof is grass thatched. This provides cool temperatures inside huts just like in local houses. Each experimental hut has one door and four windows. The huts have open spaces (eaves) between the roof and the wall similar to local huts. This results in volume, surface area, temperature and air-flow profiles similar to local homes, which is extremely important when measuring spatially active vector control tools. Half of the eaves and all of the windows are fitted with exit traps suspended outside the huts to trap those mosquitoes that attempt to leave. The traps are made of metal frames and UV resistant black plastic coated fibreglass netting (Phifer, USA). The traps are fitted with cotton sleeves through which mosquitoes can be collected. On the eaves there are spaces left between traps. These spaces are fitted with netting baffles through which mosquitoes enter huts but cannot leave. Mosquitoes can only leave through exit traps. Previous studies indicated that entry behaviour of mosquitoes in experimental huts was similar to local houses [17].

Study design

A partially-randomized fully-balanced 4×4 Latin square design was performed to determine efficacy of DDT used as IRS, Transfluthrin and Metofluthrin coils in four experimental huts. The treatments were tested for four nights per week and were rotated weekly. Therefore, one balanced round of experiments was completed in 16 days. Four rounds of 16 days were performed ($n = 64$ nights). The treatments tested were: 1) standard control – DDT IRS; 2) negative control – no insecticide used; 3) two Transfluthrin coils (0.03%) per hut each night and 4) two Metofluthrin (0.00625%) coils per hut each night. The huts were located approximately 300 metres from local houses and arranged linearly along a mosquito-breeding site with 50 metres spaces left between them to minimize interaction between treatments. Treatments were randomly allocated to huts with two male volunteers. Treatments were not moved between huts on a nightly basis because of the possibility of a carryover effect of treatments. The huts were left without treatments during the fifth, sixth and seventh night in order to wash out the effect of the previous treatment, after which treatments were moved to the next hut. Two coils were placed on the floor in the middle of the hut at the start of the experiment and they were replaced with new ones when they burnt out. Freshly sprayed DDT mats were used for each round of experiment, meaning that sprayed mats were used for one month and kept in a store to be later burnt in an incinerator.

Mosquito collection

Experiments were conducted between 24th November 2010 and 15th October 2011 for 64 nights. Experiments took place each night between 1800 hours and 0600 hours. Every evening, volunteers removed all insects and predators from exit traps to prepare huts for the next experimental night and then they retired to bed. In huts selected for coils, the technicians lit two coils and volunteers were given additional coils and instructed to replace those that burnt out before 0600 hours. Volunteers slept under untreated bed nets and woke up at the top of every hour to collect mosquitoes from exit traps. Mosquitoes were collected between 1900 hours and 0600 hours using a mouth aspirator and a spotlight for a maximum of 15 minutes each hour. At 0600 hours, all mosquitoes resting inside the huts as well as those found on the floor were collected. Mosquitoes were placed in paper cups labelled by the time and place of collection (exit traps, resting on hut surfaces and the floor), provided with 10% glucose solution soaked on pieces of cotton wool and kept in a field insectary for 24 hours. Mean temperature inside the insectary was $29.1^{\circ}\text{C} \pm 3.0^{\circ}\text{C}$ during the day and $26.7^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$ at night, while mean relative humidity was $70.6\% \pm 17.9\%$ during the day and $75.7\% \pm 13.7\%$ at night. The insectary was located 50 m away from experimental huts.

Mosquito handling and identification

Each morning, mosquitoes previously collected from huts and kept for 24 hours in the insectary were morphologically identified as *An. gambiae s.l.*, *Mansonia* spp. or *Culex* spp. Mosquitoes were also grouped as dead, alive, fed or unfed. A sub sample of the *Anopheles*

genus mosquitoes was randomly selected and transported to the laboratory for further identification to species using ribosomal DNA-polymerase chain reaction (PCR) [20].

Quality control: assessment of the carryover effect of airborne insecticides

During experiments, there was a three-day wash out period after four days of experiments when there were no insecticides in the huts. Volunteers entered huts at 1800 hours and slept until 0600 hours. They collected mosquitoes in exit traps, from resting surfaces inside huts and the floor at 0600 hours. This experiment enabled us to determine whether the three-day wash out period was sufficient to reduce any residual airborne insecticides before treatments were rotated between huts.

Assessment of residual efficacy of DDT on grass woven mats

The method of evaluating residual efficacy of DDT on grass woven mats was based on the WHO insecticide testing guidelines [16]. Two locations on each of the four “walls” of DDT sprayed mats were randomly selected. We attached WHO cones on the walls using masking tape and 10 laboratory-reared, 2–6 day old female nulliparous *An. arabiensis* mosquitoes were introduced into each cone. The time was noted and mosquitoes were removed from the cones after 30 minutes. Mosquitoes removed from cones were kept in the field insectary and monitored for 24 hours after which dead and live mosquitoes were recorded. Bioassays were conducted a day after spraying and once every week for four weeks during

experiments. Additional control bioassays were conducted simultaneously on control mats previously sprayed with water only.

4.3.4. Experiment 2: semi-field

Semi-field system

Studies were conducted in experimental huts placed inside a Semi-Field System (SFS) in Bagamoyo District, Tanzania (Figure 4.2) Use of the SFS [14] allowed replications of experiments within a short period of time because laboratory reared mosquitoes were used and therefore experiments were not dependent on the season. In addition, laboratory mosquitoes are disease free, therefore, not putting volunteers at risk of being infected with mosquito-borne diseases.



Figure 4.2. Semi-field system. The walls and the roof of the semi-field system (SFS) are made of metal frames and fibreglass netting material. It was divided into four equal square sections divided by fibreglass netting. An experimental hut was placed in each compartment. The SFS [14] allowed replication of experiments within a short period of time. Laboratory reared mosquitoes were used and were available throughout the duration of experiments hence there were no delays as usually experienced in the field.

Mosquitoes

Insecticide susceptible mosquitoes of the species *An. gambiae s.s.* (Ifakara strain) were used. The colony was maintained by feeding larvae on Tetramin fish food and adults on human blood between 3 and 6 days after emergence and 10% glucose solution *ad libitum*. Temperature and humidity within the insectary were maintained between 28 – 29°C and 70 – 80% respectively. The mosquitoes used in the experiments were female nulliparous, 3–8 days old *An. gambiae s.s.* that had never blood fed and were sugar starved for 6 hours prior to the start of experiments.

Study design

Four Ifakara design experimental huts (Figure 3.2) fitted with window and eave exit traps were used inside the SFS. The huts were placed in individual compartments separated by 10 metres and a netting screen. A fully-randomized fully-balanced 4×4 Latin square design was performed to determine efficacy of DDT used as IRS, Transfluthrin and Metofluthrin coils in four experimental huts. The treatments were tested for four nights per week. Therefore, one balanced round of experiments was completed in 16 days. The treatments tested were: 1) standard control – DDT as IRS; 2) negative control – no insecticide used; 3) two Transfluthrin coils (0.03%) per hut each night and 4) two Metofluthrin (0.00625%) coils per hut each night. Treatments and two male volunteers were randomly allocated to each hut. The pair of volunteers was rotated between huts every fourth night while the treatments remained in the same huts during the entire study period. Equal numbers of

mosquitoes were used in each compartment; hence there was no need to rotate the treatments between huts to minimize location bias as is the case in field experiment. Experiments began each evening at 1930 hours when volunteers entered respective huts. Technicians placed two lit coils on the floor 0.5 m from the volunteer inside respective huts (Figure 4.3). After 10 minutes, the volunteers simultaneously released 100 female mosquitoes in each hut from netting cages. The volunteers slept on mattresses on the floor and did not use bed nets.



Figure 4.3. Process of collecting mosquitoes from experimental huts. A: A coil placed on the floor 0.5 m from the volunteer **B:** HN collecting mosquitoes from exit traps using a mouth aspirator; **C:** AM collecting resting mosquitoes using a backpack aspirator; **D:** HN sorting mosquitoes and keeping them in individual tubes for checking oviposition.

Mosquito collection and processing

Technicians collected mosquitoes from exit traps at the top of every hour from 2100 hours to 0700 hours using mouth aspirators (Figure 4.3 B). Additional collection was done at 0700 hours inside the huts to capture resting, knocked down and dead mosquitoes using CDC backpack aspirators (Figure 4.3. C). Mosquitoes were placed in labelled paper cups and provided with 10% glucose solution. They were kept in an insectary with temperature at 28 – 29°C and between 70 – 80% relative humidity. Each morning mosquitoes were sorted as either dead or alive, and fed and unfed. The total number of mosquitoes in each group was recorded. Blood fed mosquitoes were kept in the insectary in individual vials with moist filter paper and were left to lay eggs (Figure 4.3.D). The number of eggs in each vial was counted and recorded after 3 days.

Protection of participants and ethical approval

The male persons who slept in experimental huts were recruited on a voluntary basis through written informed consent after the risks and benefits of the study were clearly explained, and they were free to leave at any time during the study. The participants were screened for malaria before the beginning of the study and those participants found malaria positive were given artemisinin combination therapy anti-malarial drugs and referred to the nearest health centre. Those fit to participate in the study were tested for malaria every two weeks. Adverse events such as respiratory symptoms were monitored. The participants were also compensated for their time and effort. The ethical review boards of Ifakara Health Institute IHI/IRB/No A-019-2007, the National Malaria Research Institute Tanzania

(NIMR/HQ/R.8a/Vol.1X/710) and the London School of Hygiene and Tropical Medicine (LSHTM ERB 5552) approved the study.

4.3.5. Statistical analysis

In experiment 1, the mortality of mosquitoes in the field was calculated using the WHOPES formula due to the low number of dead mosquitoes collected, while deterrence (Experiment 1), contact irritancy and excito-repellency, feeding inhibition and fecundity, (Experiment 2) were analyzed using the R statistical software version 3.02 [21] with a significance level of 0.05 for rejecting the null hypothesis. All generalized linear mixed models (GLMMs) were conducted using the lme4 package [22].

Assessment of residual efficacy of DDT on grass woven mats

Mortality of mosquitoes in different cone assays was calculated as a proportion of the total number of those exposed to the chemical.

Deterrence

Deterrence was determined using GLMMs. The model included the number of mosquitoes as the response variable (dependent variable) and the independent variables included the hut (because only 4 huts were used in the study) and treatment as fixed factors and the day of experiment as a random variable. The first model did not account for overdispersion in the

data (performing a Poisson GLMM), the second model accounted for overdispersion by fitting a random intercept for each row of the data (performing a log-normal Poisson GLMM) and the third model was fitted with an interaction term between hut and treatment and accounted for overdispersion. The models were compared using Akaike's Information Criterion (AIC) [23] and the second model was chosen because it had the smallest AIC.

Toxicity

The proportion of mortality in the field study was calculated using the following formula: $100 \times (D_t - D_c) / E_c$ (The proportion of dead mosquitoes D_t = number of mosquitoes dead in treated hut, D_c = number of mosquitoes dead in control hut and E_c = total number of mosquitoes in control hut. This formula is used to calculate the overall insecticidal effect of treatment inside huts [16]). Mortality in the semi-field studies was determined by fitting a GLMM with binomial error and a logit link function. The dependent variable was the proportion of dead mosquitoes and independent variables were treatment and trap (exit or floor or resting) included as fixed factors while the day of experiment was set as a random variable.

Contact irritancy and excito-repellency

The number of mosquitoes that exited huts was compared to those that stayed inside the huts that had insecticides relative to the control. A GLMM with a binomial error and a logit link function was fitted. The dependent variable was the proportion of exiting mosquitoes.

Independent variables included treatment as fixed factor and day as a random factor.

The rate at which mosquitoes left huts that had insecticides was compared to the control huts using survival analysis and Kaplan-Meier survival graphs. Analysis was conducted with survival and splines survival packages in R 3.02 [21]. The time at which an individual mosquito left the hut was considered to be the “event”.

Blood feeding inhibition

The proportion of blood-fed mosquitoes was compared between the treatment and control huts in the semi-field experiments. This was determined by fitting a GLMM with binomial error and logit link. The dependent variable was the proportion of unfed mosquitoes and independent variables included treatment, volunteer and trap type as fixed factors and day as a random variable.

Reduced fecundity

The data was analysed in two different ways. The first method was to determine the proportion of mosquitoes that laid eggs after blood feeding in the presence of insecticides in semi-field experiments. This was determined by fitting a GLMM with binomial error and logit link. Treatment was included as a fixed factor and day of experiment as a random variable.

The second method was used to determine the number of eggs laid by blood fed mosquitoes exposed to insecticides compared to the control. The effect on number of eggs laid was determined using a GLMM. A Poisson model was fitted with the number of eggs as the dependent variable and the independent variables included treatment as a fixed factor and the day of experiment as a random variable. The best fitting model as measured by AIC did not account for overdispersion.

4.4. Results

4.4.1. Experiment 1 field

The total number of mosquitoes collected was 30,280 of which 19,593 mosquitoes were *An. gambiae s.l.*, 2016 were *Mansonia* sp. 7829 were *Culex quinquefasciatus*, 136 were *Stegomyia aegypti* [24] and 706 were *An. coustani*. PCR analysis was conducted on species of *An. gambiae s.l.*, 100% (n = 975) of all successful amplifications were *An. arabiensis* mosquitoes [25].

Quality control: assessment of the carryover effect of airborne insecticides

During the three-day wash period, the total number of mosquitoes inside huts increased gradually from the first day to the second day but there was no significant difference between the first day and the last two days (Table 4.2). There was no significant difference in the number of mosquitoes between huts that previously contained insecticides and the control hut (Table 4.3).

Table 4.2: Total mosquitoes collected from experimental huts in the field during the 3 – day wash out period (experimental nights; n = 12).

| Day of wash out | N | Median | IQR | Odds | Odds Ratio | z Value | p Value |
|-----------------|------|--------|----------------|-------|------------|---------|---------|
| 1 | 1064 | 42.00 | 22.80 – 91.80 | 48.92 | 1.00 | NA | NA |
| 2 | 1232 | 46.50 | 37.50 – 123.00 | 54.73 | 1.12 | 0.237 | 0.812 |
| 3 | 1187 | 71.00 | 33.30 – 92.30 | 59.61 | 1.22 | 0.418 | 0.676 |

This table illustrates the indoor densities of mosquitoes of experimental hut that previously had coils and DDT. Entry of mosquitoes was measured for 3 days. N - Total number of mosquitoes; Median – Number of mosquitoes per experimental day; IQR – Interquartile range.

Table 4.3: Total mosquitoes that entered untreated huts that previously had insecticides (experimental nights; n = 12)

| Treatment | N | Median | IQR | Odds | Odds ratio | z Value | p Value |
|----------------------|----------|---------------|----------------|-------------|-------------------|----------------|----------------|
| No insecticide | 1048 | 47.00 | 22.00 – 110.30 | 53.67 | 1.00 | NA | NA |
| Transfluthrin coils | 737 | 71.00 | 28.00 – 92.50 | 53.70 | 1.00 | 0.006 | 0.995 |
| Metofluthrin coils | 877 | 67.00 | 39.80 – 103.00 | 61.02 | 1.14 | 1.220 | 0.223 |
| DDT 2gm ² | 821 | 42.50 | 37.50 – 72.30 | 49.10 | 0.91 | -0.832 | 0.406 |

This table illustrates the indoor densities of huts that previously had coils and DDT. The mosquitoes were collected during the wash period when the huts had no insecticides. N = Total number of mosquitoes; Median = Number of mosquitoes per hut per night; IQR – Interquartile range

Deterrence

All compounds deterred malaria vectors from entering huts but coils had a greater impact than DDT (Table 4.4). Transfluthrin coils reduced entry of *An. arabiensis* mosquitoes by 38% (RR – 0.62 [0.47 - 0.87]; $z = -6.37$, $p < 0.001$) and Metofluthrin coils reduced *An. arabiensis* mosquitoes by 30% (RR – 0.70 [0.50 - 0.98]; $z = -4.77$, $p < 0.001$) (Table 4). Both Metofluthrin and Transfluthrin coils reduced entry of *Mansonia* spp. mosquitoes by more than three quarters while DDT reduced them by half (Table 4.4). There was no significant difference in the number of *Cx. quinquefasciatus* mosquitoes entering control, DDT and Transfluthrin huts although Metofluthrin coils did reduce their entry.

Table 4.4: Indoor mosquito densities in field experimental huts that had mosquito coils and DDT compared to huts that did not have insecticides (n=64 nights).

| Treatment | N | Median | IQR | RR | 95% CI | z Value | p Value |
|-------------------------------|------|--------|----------------|------|---------------|---------|---------|
| <i>Anopheles arabiensis</i> | | | | | | | |
| No insecticide | 5650 | 70.00 | 50.25 – 104.50 | NA | NA | NA | NA |
| Transfluthrin n coils | 3881 | 47.00 | 27.25 – 75.25 | 0.62 | [0.47 - 0.87] | -6.37 | <0.001 |
| Metofluthrin coils | 4249 | 54.00 | 35.50 – 82.00 | 0.70 | [0.50 - 0.98] | -4.77 | <0.001 |
| DDT 2gm ² | 5813 | 67.00 | 41.50 – 108.75 | 0.92 | [0.65 - 1.20] | -1.22 | 0.224 |
| <i>Culex quinquefasciatus</i> | | | | | | | |
| No insecticide | 2300 | 26.00 | 19.50 – 46.25 | NA | NA | NA | NA |
| Transfluthrin n coils | 1782 | 26.50 | 13.00 – 39.25 | 0.87 | [0.73 - 1.05] | -1.46 | 0.143 |
| Metofluthrin coils | 1645 | 22.50 | 13.75 – 36.25 | 0.72 | [0.61 - 0.85] | -3.80 | <0.001 |
| DDT 2gm ² | 2102 | 27.00 | 16.75 – 44.00 | 1.13 | [1.01 - 1.28] | -1.40 | 0.161 |
| <i>Mansonia spp.</i> | | | | | | | |
| No insecticide | 947 | 12.00 | 8.75 | NA | NA | NA | NA |
| Transfluthrin n coils | 150 | 2.00 | 1.00 | 0.16 | [0.07 - 0.19] | -8.17 | <0.001 |
| Metofluthrin coils | 185 | 2.00 | 0.75 | 0.12 | [0.09 - 0.24] | -7.56 | <0.001 |

| | | | | | | | |
|----------------------|-----|------|------|------|---------------|-------|-------|
| DDT 2gm ² | 734 | 9.00 | 5.75 | 0.50 | [0.33 - 0.77] | -3.16 | 0.002 |
|----------------------|-----|------|------|------|---------------|-------|-------|

N- Total number of mosquitoes; Median – number of mosquitoes per hut per night; IQR – Interquartile range; RR – Relative rate; CI – Confidence intervals

Toxicity

Mortality of mosquitoes after 24 hours in field experiments was very low. Only 0.02% mortality of all mosquito species collected was observed.

Residual efficacy of DDT on grass woven mats

Cone bioassays conducted on DDT mats on the second day and a week after spraying showed 100% mortality of mosquitoes after 24 hours. Mortality dropped in the second, third and fourth week to 73%, 92% and 90%, respectively. It is likely that DDT flaked off from mats when they were moved between huts resulting in reduced residues hence reduced toxicity. There was no mortality in the bioassays conducted on control mats.

4.4.2. Experiment 2: semi-field

Seventy percent ($n = 4476/6400$) of the mosquitoes released in the huts were recaptured. The relatively low recovery rate could be explained by loss of mosquitoes that might have been eaten by predators and those that escaped through small cracks in the huts or when the door was opened briefly. However analysis was conducted on recovered mosquitoes and not released mosquitoes.

Contact irritancy and excito-repellency

The proportion of mosquitoes that left huts that had DDT, Transfluthrin and Metofluthrin coils was significantly higher than the control (Table 4.5).

Approximately 48% (95% CI: [0.44 -0.53]; $z = 9.950$, $p < 0.001$) of the mosquitoes left DDT huts (Table 4.5). In huts with Transfluthrin and Metofluthrin coils approximately 56% (95% CI: [0.51 - 0.60]; $z = 12.779$, $p < 0.001$) and 55% (95% CI: [0.51 -0.60]; $z = 12.890$, $p < 0.001$) mosquitoes left huts, respectively. The rate at which mosquitoes left huts throughout the night is illustrated using Kaplan Meier survival curves (Figure 4.4). The highest exodus of mosquitoes from huts was observed in the first half of the night (2100 – 0000 hours) regardless of treatment or control, but overall, more mosquitoes exited when huts contained DDT, Transfluthrin or Metofluthrin coils compared to the control.

Table 4.5: The proportion of mosquitoes that left experimental huts that had mosquito coils and DDT compared to the hut that did not have insecticides in the semi field system (experimental hut nights = 16).

| Treatment | Proportion of mosquitoes^a | OR [95% CI] | Mean proportion | 95% CI | z value | p value |
|----------------------------|-------------------------------------------------|--------------------|------------------------|---------------|----------------|----------------|
| Control | 313/1067 | 1.00 [0.00 - 2.00] | 0.27 | [0.22 - 0.34] | -6.68 | NA |
| DDT | 581/1185 | 2.32 [0.45 – 4.55] | 0.48 | [0.44 -0.53] | 9.95 | <0.001 |
| Transfluthrin coils | 599/1067 | 3.08 [0.61 - 6.08] | 0.56 | [0.51 - 0.60] | 12.78 | <0.001 |
| Metofluthrin coils | 645/1157 | 3.03 [0.63 - 5.98] | 0.55 | [0.51 -0.60] | 12.89 | <0.001 |

a = mosquitoes in exit traps/total number of mosquitoes recaptured from the hut; CI – Confidence intervals CI – Confidence intervals; OR – Odds ratios of the proportion of mosquitoes

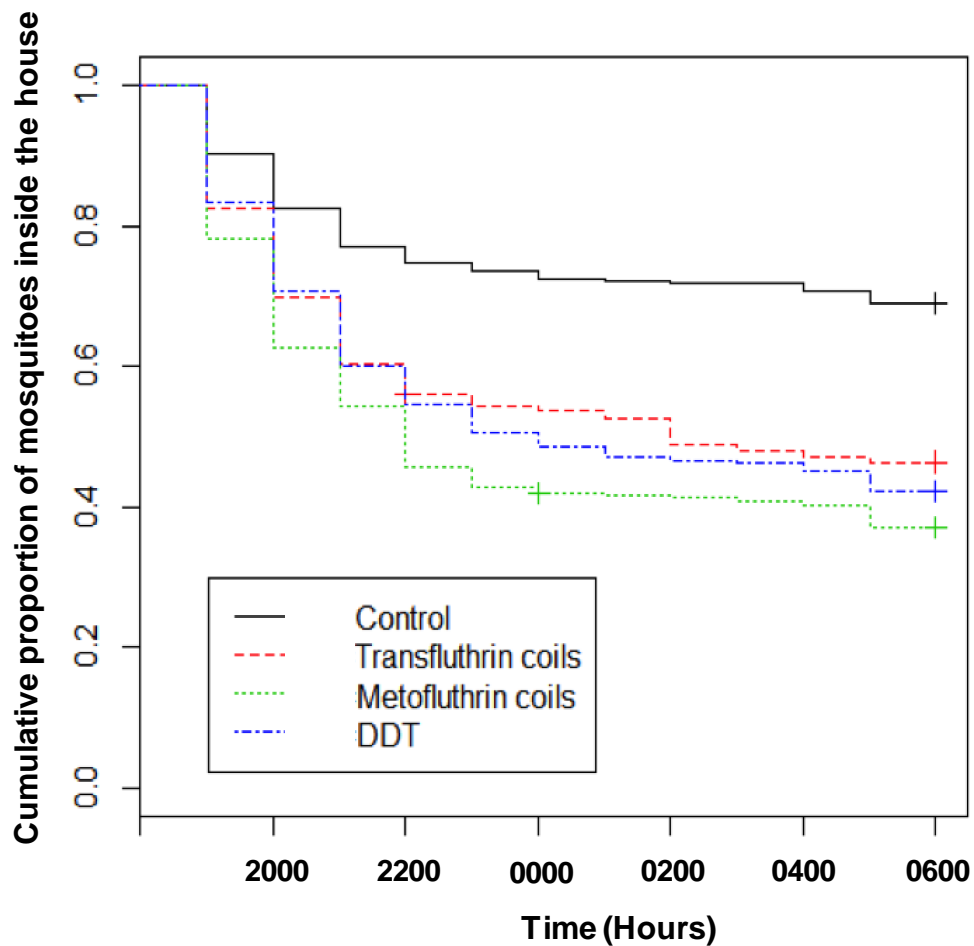


Figure 4.4. Survival curves illustrating the rate at which mosquitoes left huts with DDT, transfluthrin and metofluthrin coils. The curves represent the rate at which mosquitoes exit huts that have different insecticides compared to the control. Analysis was based on a Kaplan-Meier stepped survivorship function. Each curve represents one treatment.

Toxicity

The proportion of mortality in control huts was 18% ($n = 193/1067$). Therefore, Abbot's correction formula was used to correct for mortality induced by tested insecticides because mortality in the control huts was more than 10% [15]. There was a much higher proportion of mortality induced by insecticides in the semi-field study compared to the field. DDT induced 64% (95% CI: [0.60 - 0.67]; $z = 22.49$, $p < 0.001$), Transfluthrin induced 66% (95% CI: [0.63 - 0.70] $z = 23.32$, $p < 0.001$) and Metofluthrin 61% (95% CI: [0.57 - 0.64]; $p < 0.001$; $z = 21.96$) mortality (Table 4.6). More than 90% of the mosquitoes collected inside huts that had mosquito coils and DDT had died within 24 hours unlike in the control hut (Table 4.7). Out of the mosquitoes collected from exit traps of DDT, Transfluthrin and Metofluthrin huts, 49%, 46% and 57%, respectively died after 24 hours (Table 4.7).

Table 4.6: The proportion of the mortality of mosquitoes 24 hours after collection from experimental huts

| Treatment | Total dead | Total | Crude mortality | | OR [95% CI] | Corrected mortality [‡] | | z value | p value |
|----------------------------|------------|------------|-----------------|---------------|----------------------|----------------------------------|---------------|---------|---------|
| | mosquitoes | mosquitoes | Mean | [95% CI] | | Mean | [95% CI] | | |
| | | recaptured | proportion | | | proportion | | | |
| Control | 193 | 1067 | 0.17 | [0.13 - 0.22] | 1.00 [0.00 – 2.00] | 0.00 | [0.00 - 0.00] | -10.04 | NA |
| DDT | 836 | 1185 | 0.70 | [0.65 - 0.74] | 9.68 [4.19 – 21.00] | 0.64 | [0.60 - 0.67] | 22.49 | <0.001 |
| Metofluthrin coils | 763 | 1157 | 0.67 | [0.63 - 0.72] | 8.77 [2.34 – 17.79] | 0.61 | [0.57 - 0.64] | 21.96 | <0.001 |
| Transfluthrin coils | 727 | 1067 | 0.72 | [0.68 - 0.76] | 10.85 [1.53 – 21.01] | 0.66 | [0.63 - 0.70] | 23.32 | <0.001 |

Experiments were conducted in experimental huts within a semi field system. Mortality of mosquitoes is compared between huts that had mosquito coils, DDT and no insecticide. Experiments were conducted for 16 nights. CI – Confidence intervals. [‡] - Corrected using Abbott's formula; OR – Odds ratios of the proportion of mosquitoes

Table 4.7: Mortality of mosquitoes collected from exit traps compared to those collected inside experimental huts

| Treatment | Mosquitoes in exit traps | | | Mosquitoes indoors | | |
|----------------------------|--------------------------|--------|-------------|--------------------|---------------------|-------------|
| | Dead | Median | IQR | Dead | Median ² | IQR |
| | mosquitoes/Total | | | mosquitoes/Total | | |
| | mosquitoes | | | mosquitoes | | |
| Control | 91/313 | 0.35 | 0.19 – 0.43 | 102/754 | 0.15 | 0.09 -0.18 |
| DDT | 286/581 | 0.52 | 0.30 – 0.75 | 550/604 | 1.00 | 0.94 – 1.00 |
| Transfluthrin coils | 273/599 | 0.49 | 0.34 – 0.55 | 454/468 | 1.00 | 1.00 - 1.00 |
| Metofluthrin coils | 333/645 | 0.35 | 0.25 – 0.73 | 430/512 | 1.00 | 1.00 - 1.00 |

The proportion of mortality induced by insecticides was measured in experimental huts in a semi field system for 16 nights. Mortality was compared between huts that had mosquito coils, DDT and no insecticides. Median – median proportion of mosquitoes per hut per night; IQR – Interquartile range

Blood feeding inhibition

Blood-feeding inhibition was the most pronounced mode of action in all three treatments. Transfluthrin and Metofluthrin coils had the highest impact on feeding of mosquitoes. Transfluthrin coils reduced feeding by 98% (95% CI: [0.96 - 0.99]; $z = 22.03$, $p < 0.001$), Metofluthrin reduced it by 93% (95% CI: [0.90 - 0.95]; $z = 25.57$, $p < 0.001$) and DDT by 77% (95% CI: [0.73 - 0.81]; $z = 24.10$, $p < 0.001$) (Table 4.8).

Table 4.8: Insecticide induced blood-feeding inhibition of mosquitoes in experimental huts

| Treatment | Proportion of unfed mosquitoes ^a | OR [95%CI] | Mean proportion | [95% CI] | z value | p value |
|----------------------------|----------------------------------------------------|-------------------------|------------------------|-----------------|----------------|----------------|
| Control | 321/1120 | 1.00 [0.00 – 2.00] | 0.15 | [0.10 - 0.22] | -7.49 | NA |
| DDT | 881/1047 | 13.21 [9.96 – 29.04] | 0.77 | [0.73 - 0.81] | 24.10 | <0.001 |
| Transfluthrin coils | 1164/1184 | 144.87 [67.19 – 382.05] | 0.98 | [0.96 - 0.99] | 22.03 | <0.001 |
| Metofluthrin coils | 1085/1146 | 44.27 [37.03 – 110.97] | 0.93 | [0.90 – 0.95] | 25.57 | <0.001 |

The proportion of mosquitoes that were inhibited from blood feeding on humans was measured inside experimental huts inside a semi field system for 16 nights. The proportion of unfed mosquitoes was compared between mosquito coils, DDT and no insecticide. ^a - unfed mosquitoes /total number of mosquitoes recaptured from the hut; OR – Odds ratios of the proportion of mosquitoes that are likely not to feed; CI – Confidence intervals

Reduced fecundity

The difference in the number of mosquitoes that laid eggs versus those that did not lay eggs was determined from the number that acquired blood meals. The proportion of mosquitoes that laid eggs was low in all huts (Table 4.9). There was no difference in the proportion of mosquitoes that laid eggs between treatments relative to the control. DDT reduced the total number of eggs laid per female by 90% (RR – 0.10 [0.04 - 0.26]; $z = -4.57$, $p < 0.001$), Transfluthrin coils by 97% (RR – 0.03 [0.01 - 0.15]; $z = -4.13$, $p < 0.001$ and Metofluthrin coils by 91% (RR – 0.09 [0.03 - 0.27]; $p < 0.001$; $z = -4.28$) (Table 4.10).

Table 4.9: The fecundity of mosquitoes after exposure to mosquito coils and DDT in experimental huts

| Treatment | Total mosquitoes that laid eggs /Total blood fed mosquitoes | OR [95%CI] | Mean proportion | [95% CI] | z value | p value |
|----------------------------|--------------------------------------------------------------------------------|--------------------|----------------------------|-----------------|----------------|----------------|
| Control | 202/614 | 1.00[0.00 – 2.00] | 0.33 | [0.28 - 0.37] | -7.36 | NA |
| DDT | 19/76 | 0.68[-0.32 – 1.41] | 0.20 | [0.13 - 0.30] | -2.41 | 0.016 |
| Transfluthrin coils | 1/6 | 0.41[-0.36 – 3.02] | 0.15 | [0.02 - 0.61] | -0.94 | 0.347 |
| Metofluthrin coils | 11/34 | 0.96[-0.57 – 5.98] | 0.24 | [0.13 - 0.40] | -1.13 | 0.258 |

Legend: Fecundity was measured by determining the proportion of mosquitoes that laid eggs out of those that successfully blood fed. Fecundity was compared between mosquitoes exposed to mosquito coils, DDT and no insecticide inside experimental huts in the semi field system for 16 nights.
CI – Confidence intervals; OR – Odds ratios of the proportion of mosquitoes

Table 4.10: The number of eggs laid by mosquitoes collected from experimental huts

| Treatment | Total number of eggs | Median | IQR | RR | [95% CI] | z Value | p Value |
|----------------------------|-----------------------------|---------------|---------------|-----------|-----------------|----------------|----------------|
| Control | 10089 | 649.0 | 443.5 - 943.0 | NA | NA | 18.66 | NA |
| Transfluthrin coils | 57 | 0.0 | 0.0 - 0.0 | 0.03 | [0.01 – 0.15] | -4.13 | <0.001 |
| Metofluthrin coils | 526 | 0.0 | 0.0 - 57.5 | 0.09 | [0.03 - 0.27] | -4.28 | <0.001 |
| DDT 2gm² | 837 | 42.0 | 6.0 - 82.5 | 0.10 | [0.04 - 0.26] | -4.57 | <0.001 |

Blood fed mosquitoes collected from huts that had mosquito coils, DDT and no insecticides were kept in individual oviposition tubes and the number of eggs laid was compared between mosquitoes that had been collected from huts that had different insecticides. The number of eggs was fitted in a Poisson model because the number of eggs was count data. IQR – Interquartile range; RR – Relative rate; CI – Confidence intervals

4.5. Discussion

Traditionally, efficacy of insecticides for disease control is attributed to toxicity while other effects are considered less important. The spread of insecticide resistance threatens the sustainability of insecticides applied to kill mosquitoes [26, 27]. While development of new insecticides is an undisputed requirement to fight insecticide resistance, management of existing insecticides to prolong their usefulness is also necessary.

A critical look at the modes of action of insecticides by several authors indicate that toxicity may not be the single most important action of insecticides as far as malaria transmission is concerned [7, 10, 28, 29]. Experimental hut studies enable detailed observation of the impact of insecticides on mosquito behaviour [30, 31]. This study substantiates the mode of action of reduced blood feeding by mosquitoes [9] and irritancy [7, 32] (Figure 4.5). It is worth noting that despite the irritant effect of chemicals, 49% 46% and 57% of the mosquitoes that left DDT, Transfluthrin and Metofluthrin huts respectively died after 24 hours (Table 4.7). Moreover this study shows that the magnitude of these effects was similar between coils and DDT (Figure 4.5).

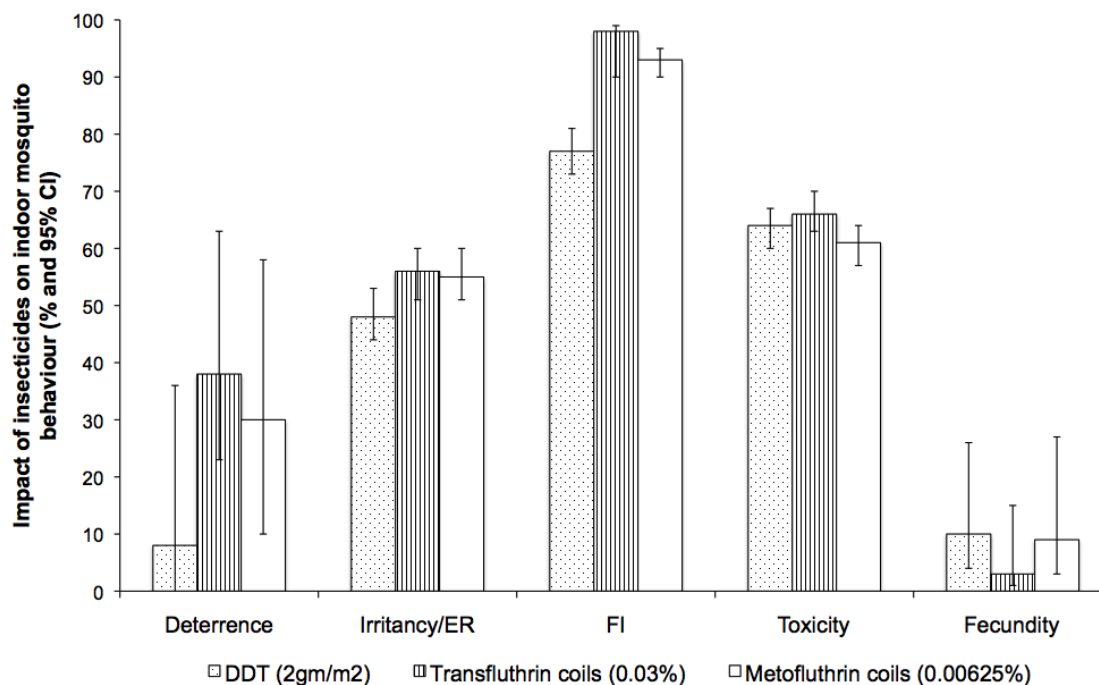


Figure 4.5. Overall impact of insecticides on mosquito behaviour inside houses. The graph illustrates the mode of action of DDT, Transfluthrin and Metofluthrin coils on mosquito behaviour. The outcomes measured included deterrence, irritancy, feeding inhibition and toxicity. The value of deterrence was derived from the effect of insecticides on *An. arabiensis* mosquitoes from field experiments and irritancy, feeding inhibition, mortality and fecundity of *An. gambiae* s.s. mosquitoes from the semi field system experiment.

Using figures collected from the field (deterrence) and the semi field experiments (irritancy, feeding inhibition, mortality and fecundity) it can be seen that in a scenario where 100 mosquitoes approach a house, deterrence comes into play in the first instance and only approximately 62, 70 and 92 mosquitoes enter the house with Transfluthrin, Metofluthrin coils and DDT respectively. The next behavioural effect of the insecticides is then likely to be irritancy or excito–repellency. After mosquitoes are repelled and exit a house, 35, 39 and 44 would remain inside the house with Transfluthrin, Metofluthrin coils and DDT respectively. Of those, approximately 1, 3 and 10 mosquitoes would manage to acquire a blood meal, which in turn directly influences the proportion of eggs laid,

i.e. female mosquito fecundity. Lastly, the survival rate of mosquitoes in Transfluthrin and Metofluthrin huts would be close to 0 and approximately 10 in DDT huts (Figure 4.6). This implies that through deterrence, irritancy and feeding inhibition of pyrethroid coils and DDT, more than 90% of the mosquitoes would be prevented from contacting humans inside houses before mortality is even considered. By reducing human-vector contact, coils and DDT directly influence the biting rate of mosquitoes (ma): an important parameter of malaria transmission Vectorial capacity equation, subsection). The data collected on DDT, agrees with field observations [9] of feeding inhibition and population level data that consistently demonstrate a reduction in the Human Blood Index (HBI) after DDT is applied to dwellings [33, 34]. However, the experimental design does have the limitation of combining data from two species: *An. arabiensis* and *An. gambiae* s.s.

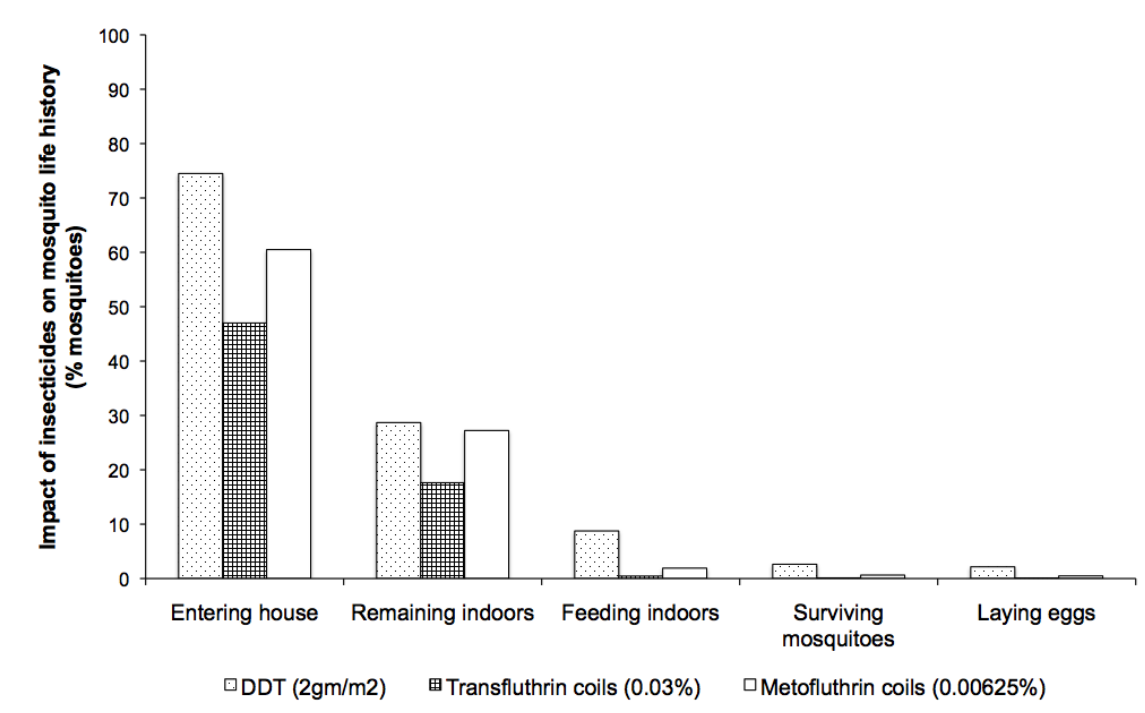


Figure 4.6. Impact of insecticides on mosquito behaviour around and insides houses. The graph illustrates the effect of DDT, Transfluthrin and Metofluthrin coils on the house entry and behaviour of 100 female *An. arabiensis* and *An. gambiae* s.s. mosquitoes are approaching the house. Assumptions made included the fact that deterrence was the first mode of action followed by irritancy, feeding inhibition, toxicity and fecundity. The data used was derived from field experiments for deterrence and semi-field system experiments for irritancy, feeding inhibition, toxicity and fecundity.

Studies have been conducted on the host preference and time and place of biting and resting in Kilombero. It is known that *An. arabiensis*, the dominant *Anopheles* species in Kilombero, readily enter houses [17], and exit to rest outside whereas *An. gambiae* s.s. feed and rest indoors (K. Kreppel, unpublished). The human blood index (HBI) of *An. arabiensis* is related to the availability of human hosts, and since cattle are not common in Kilombero due to the Tanzanian Government

forcibly relocating Pastoralists and their 250,000 cattle, *An. arabiensis* feeds almost exclusively on humans in the area, indoors and outdoors (K. Kreppel, unpublished). As the impact of spatial repellents indoors was being measured, a standard laboratory strain of *An. gambiae* s.s. for repellent testing was used [15]. Previous unpublished work in local houses demonstrated that *An. arabiensis* demonstrated a similar response to 0.03% Transfluthrin coils as that measured in experimental huts with >95% feeding inhibition as measured by human landing catch. It is possible that mortality data was overestimated because *An. arabiensis* might be more likely to leave treated huts than *An. gambiae* s.s., although the vast majority of *An. gambiae* s.s. in the semi-field did leave experimental huts unfed and subsequently died. It would be worthwhile to repeat the study with *An. arabiensis*, mosquitoes.

Coils and DDT induced more than two-thirds mortality of mosquitoes in the semi-field experiments compared to about 2% in the field. The mortality (18%) observed in control huts may be attributed to poor handling of mosquitoes during collection. Resting mosquitoes were collected using backpack aspirators that may have caused mechanical damage to mosquitoes and increased mortality. However, mortality in the treatments was corrected using Abbots formula. Higher mortality observed in semi-field experiments compared to the field experiments may be due to the fact that in the semi-field studies, volunteers did not sleep under bed nets and were consequently more attractive to host seeking mosquitoes that spent more time around the host trying to feed. In the field where volunteers were protected by untreated bed nets mosquitoes may have given up and left the huts. It is

possible that availability of an unprotected host and the need to obtain blood outweighs the irritant or excito-repellency effects of insecticides, meaning that mosquitoes spend more time in the house trying to obtain a blood meal, hence acquire more lethal insecticides. These observations provide useful insights for malaria control programmes and demonstrate that spatial repellents are useful for locations where people do not use nets for cultural reasons [35] or where vectors bite before people go to bed [36, 37]. The mortality of mosquitoes induced by coils was as high as that of DDT. More than 60% of the mosquitoes collected from huts after exposure to coils died within 24 hours, having acquired lethal doses. This has implications for vector control programmes as it is thought that irritancy or excito-repellency of insecticides used on LLINs attenuates efficacy by preventing contact of mosquitoes with treated surfaces [38, 39]. In this study it is shown that coils are capable of dispensing lethal doses of airborne insecticides and have the potential to reduce mosquito densities (m) and indirectly reduce chances that a mosquito would survive (p) long enough to become infectious. This study also shows that airborne pyrethroids reduce fitness of mosquitoes by reducing the number of eggs laid. Reduced fecundity is an indirect measure of pyrethroids on mosquito densities (m). However, further studies will be performed to investigate the combined impact (additional or deleterious) of indoor spatial repellents combined with LLINs on mosquito mortality and feeding success. Among challenges facing malaria control, insecticide resistance could be considered top of the list. In this particular study area susceptibility of *An. arabiensis* mosquitoes is within the WHO set range of 80% - 97% at which

resistance is suspected [40]. Therefore low mortality observed in the field could be attributed to slow emerging resistance [41-43]. A study carried out in Benin indicated that coils were effective against highly *kdr* resistant *Cx.*

quinquefasciatus quinquefasciatus (Raphael Nguessan *pers. comm*). This indicates that spatial repellency may still provide protection where resistance has developed because airborne pyrethroids have an olfactory mode of action at low concentrations [44], different from the sodium channel target. These data warrant further investigation to see whether pyrethroid-resistant mosquitoes react differently to spatial repellents in ways that would affect vectorial capacity and malaria transmission.

The risk of mosquitoes being diverted to non-users of spatial repellents is likely to be increased if mosquitoes are prevented from feeding and continue host seeking [45]. A recent study has shown that topical repellents increase the proportion of mosquitoes to nearby non-users by approximately 4 times [46]. Nevertheless, the high toxicity of coils observed in the semi-field study might contribute to community protection. Toxicity coupled with the spatial activity of coils conferring protection in a defined area, may minimize the risk to non-users. In addition, almost half of the mosquitoes that left huts with mosquito coils and DDT died after 24 hours, consequently minimizing the population of mosquitoes that would be diverted to non-users within a community.

Nevertheless, it is necessary to improve delivery formats of airborne insecticides with the aim of expanding protection to a household or a community. In addition, it is essential to quantify the effect of using spatial repellents among non-users at

different coverage levels and determine the implications on malaria transmission at a community level through large-scale trials before they are considered as a public health intervention.

Effectiveness of any vector control tool is influenced by whether or not it protects users against nuisance bites. Results from this study indicate that only Metofluthrin coils reduced house entry of *Cx. quinquefasciatus* mosquitoes by almost 28% in Lupiro village while DDT and Transfluthrin coils had no effect. The impact of all compounds on the entry of *Mansonia* spp. mosquitoes was outstanding (Table 4.4). All compounds reduced entry by more than 50%. It is necessary to develop spatial repellents that are equally effective against nuisance mosquito species in order to enhance compliance.

It should be noted that mosquito coils need to be used on a daily basis and produce smoke that could be harmful in long term exposure and might not be desirable to many people. The development of safer, effective, long lasting passive delivery formats is underway [47, 48].

4.6. Conclusion

It is critical to determine the impact of spatial repellents on malaria transmission. This study outlines several important entomological parameters that should be quantified in a proof of concept clinical trial in order to effectively determine the impact of spatial repellents on malaria epidemiology. In this study spatial repellents reduce human – vector contact and induce mortality, hence directly

affect ma , m and p which are among the most important parameters of the vectorial capacity of a mosquito population. In addition, the role of spatial repellents in integrated approach of malaria control should be critically considered with an aim of complementing existing mainstream tools. Most available control tools, such as LLINs, require daily compliance by the user and may only be fully effective where malaria vectors still bite indoors late at night. Spatial repellents may be a suitable supplementary option where mosquitoes feed in the early evening and/or rest outdoors. In addition, because they render a given space mosquito free, they will protect multiple individuals in this space. The development of a passive spatial repellent that delivers the same mosquito control benefits of the mosquito coils tested in this study, but lasts for several weeks without the need for user compliance would contribute considerably to vector borne disease prevention.

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CHAPTER FIVE

5 The mode of action of spatial repellents and their impact on vectorial capacity of *Anopheles gambiae sensu stricto*

5.1. Abstract

Malaria vector control relies on toxicity of insecticides used in bednets and indoor residual spraying only, despite evidence of reduced malaria transmission due to prevention of human – vector contact.

The overall aim of this study was to determine the impact of sub-lethal insecticides on host seeking and blood feeding of mosquitoes. Taxis boxes were used to study mosquito responses towards or away from Transfluthrin coils.

Protective distance of coils was measured in the semi-field tunnel. In addition, short-term and long-term effects of sub-lethal pyrethroids on blood feeding were measured in a semi-field tunnel and in the Peet Grady chamber. Experiments were conducted on laboratory reared susceptible *Anopheles gambiae sensu stricto* mosquitoes.

In the taxis boxes experiment a higher proportion of mosquitoes were activated and flew towards the human in the presence of Transfluthrin. Hence coils did not hinder attraction of mosquitoes towards the human but increased it. Coils reduced biting by 86% (95% CI [0.66; 0.95]) when coils were placed 0.3m around a

human in a ‘bubble’ compared to a 65% (95% CI (0.51; 0.76) reduction when coils were used as a “point source”. Results indicated that coils used, as a “bubble” were more effective than the “point source”.

Mosquitoes exposed to Transfluthrin coils in an enclosed space were prevented from feeding and only resumed normal feeding behaviour 12 hours later. However there was no effect on free flying and caged mosquitoes exposed to Transfluthrin in the semi-field tunnel.

These findings indicate that airborne pyrethroids influence mosquito behaviour in ways other than killing by minimizing human - vector contact through fewer blood feeding events. This study provides critical information necessary for the development of target product profiles of spatial repellent products that can be used to complement existing mainstream malaria vector control tools.

5.2. Introduction

The probability of mosquito vectors successfully transmitting disease pathogens to a host depends on their ability to effectively locate the host and feed. Among factors that influence the rate at which new human malaria infections are disseminated per day by a mosquito (i.e. vectorial capacity), is the man-biting rate of mosquitoes [1]. Man-biting rate describes the frequency with which mosquitoes contact humans to obtain blood. For malaria parasites to be transmitted from one person to another, mosquitoes need to feed at least twice: once to become infected and once to infect, hence it has profound effect on vectorial capacity and

consequently malaria transmission [2]. Factors that interfere with host seeking of mosquitoes influence man-biting rate. Even though man-biting rate is just one component of the vectorial capacity, it substantially contributes to variation in the stability of malaria transmission in different areas and is especially critical where vectors are anthropophilic, i.e. prefer feeding on humans [3].

The efficient malaria vectors *Anopheles gambiae sensu stricto* (s.s.) and *An. funestus* s.s have evolved innate feeding preferences for humans due to their ability to discern human kairomones from other hosts [4]. This enhances the ability of mosquitoes to locate and orient towards hosts at distances [5, 6] as far as 30 meters [7]. Several human odours have been identified as olfactory cues that govern mosquito host seeking and feeding behaviour [8, 9]. Studies of the insect olfactory system have led to identification and development of synthetic chemical compounds that attract insects to hosts [10]. This knowledge is successfully applied in the agricultural sector in the control of crop pests [11], tsetse flies [12, 13, 14] as well as mosquitoes [15, 16, 17].

Other volatile compounds, commonly known as repellents, interfere with mosquitoes' host finding ability and consequently prevent blood feeding. They are intended to reduce human-mosquito contact and lately have been shown to reduce disease transmission [18]. Repellency has been described as: 1) "taxis": - immediate directional movement of target insects such as mosquitoes away from the source of the chemical and; 2) "orthokinesis": - increased mosquito activity after contact with insecticides [19, 20]. Other studies indicate that volatile compounds such as DEET, linalool, dehydrolinalool, catnip oil and citronella

interfere with attraction of mosquitoes to host odours by blocking natural response to attractants, hence acting as attraction inhibitors and not repellents [21 ,22, 23].

On the other hand, Lucas *et al* (2007) suggest that even in the presence of airborne pyrethroids, mosquitoes are able to detect host odours but are inhibited from feeding: “When mosquitoes detected host odours, flew upwind and landed, the majority of insects were still inhibited from biting. This effect is probably a result of pyrethroid – induced neural hyperexcitation, which can occur at much lower doses than those required for insect knockdown and mortality” [24].

Mosquito behaviour elicited in response to airborne compounds including movement away from a chemical stimulus, loss of host detection, anti-feeding as well as knockdown and mortality are collectively referred to as spatial repellency.

Spatial repellents do not require physical contact of the mosquito with treated surfaces, as is the case of chemicals used in Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Nets (LLINs) but act in the volatile state at a distance.

Mosquito coils, candles and emanators impregnated with volatile pyrethroids and other compounds including plant terpenes are collectively known as spatial repellents. Among these products, coils have been most extensively studied [25] and are commonly used to control mosquitoes [26]. Coils prevent mosquitoes from entering houses, induce early exit and reduce human biting [25, 27]. Despite numerous evaluations of coils, their mode of action is not clear: Do they interfere with orientation of mosquitoes towards humans, inhibit blood feeding or even induce both processes? It is essential to ascertain which of these actions is at play to aid the development of effective spatial repellents. This study aimed to

distinguish between repellency as described by Dethier [20] and attraction inhibition [28] induced by airborne pyrethroids. The taxis box system [7] was used to measure orientation of *An. gambiae s.s.* mosquitoes towards and away from humans in the presence of airborne pyrethroids. Protective distance conferred by coils was determined in the semi-field system by measuring reduced blood feeding by mosquitoes and whether exposure of mosquitoes to different doses of pyrethroids prolonged feeding inhibition beyond the immediate effect in a way that could impede human-mosquito contact and reduce malaria transmission.

5.3. Materials and Methods

5.3.1. Test compounds

Transfluthrin coils contained a range of doses of Transfluthrin: 0.015%, 0.03% and 0.045%. Blank coils used contained no active ingredient.

5.3.2. Mosquitoes

Insecticide susceptible mosquitoes of the species *An. gambiae s.s.* Ifakara strain were used. Larvae were fed on Tetramin fish food while adults were fed on human blood between 3 and 6 days after emergence and offered 10% glucose solution *ad libitum*. Temperature within the insectary was maintained between 28 – 29°C, between 70 - 80% relative humidity and natural light periods (12:12 hours light: dark periods). Female nulliparous 3-8 days old mosquitoes that had never blood

fed and were sugar starved for 6 hours prior to starting experiments were used for all studies.

5.3.3. Experiment 1: Orientation of mosquitoes in the presence of coils and humans

Taxis boxes system

A new assay using taxis boxes to measure long-range mosquito responses to different stimuli developed at IHI [7] was used to measure effect of coils on the orientation of mosquitoes towards humans. Briefly, taxis boxes consist of three chambers measuring 40 x 40 x 40 cm separated by metal sheets: one chamber facing the stimuli, the middle chamber and one chamber facing away from the stimuli. The middle chamber has a “letter box slit” (30cm long and 2.5cm wide) on either side, which allows mosquitoes to leave the middle but reduces the likelihood of them returning. During the experimental period the sheets were lifted using a simple pulley mechanism comprising a rope and lever located 10 meters from the boxes, which opened the slits and allowed mosquitoes to fly through. The boxes were raised 15cm off the floor and placed into plastic cups with water and grease to prevent predators from entering.

Experimental design

A fully randomized study was conducted. The study involved 6 treatments; 1) a positive control – human without a coil; 2) human + blank coil; 3) human + 0.015% coil; 4) human + 0.03% coil; 5) human + 0.045% coil and 6) a negative control – no human and no coil. The last treatment was included in order to measure mosquito response in the absence of any stimulus. The response of mosquitoes was measured in taxis boxes placed 1m away from the treatment (Figure 5.1). A treatment was randomly allocated to an experimental night using the lottery method. The treatments were tested four times using four human volunteers randomly assigned on a nightly basis to give an average human response. Two taxis boxes were used to increase the sample but each box was treated as a separate factor in the analysis to ensure independence of experimental replicates.

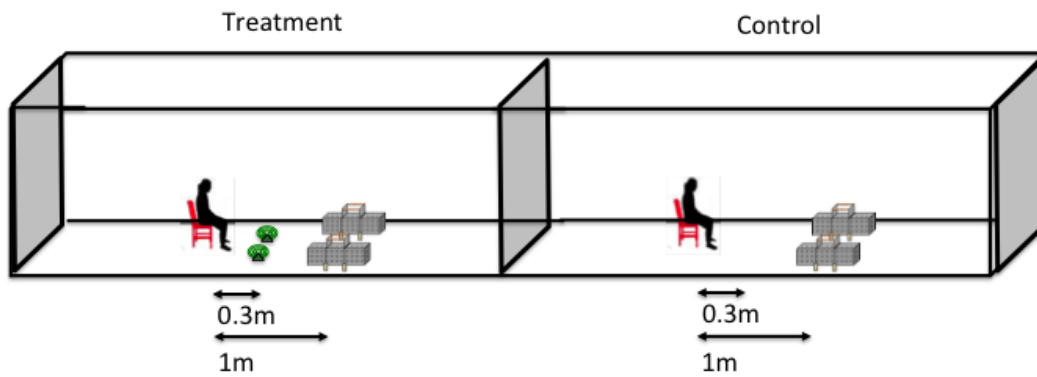


Figure 5.1: Taxis boxes experimental design

Two taxis boxes placed 1m away from the human and or human and a coil. Mosquitoes were introduced in the middle chamber of each taxis box and the stimulus was changed each day to determine the effect on orientation of mosquitoes. The semi-field tunnel measures 100m long, 2m wide and 2.5m high.

Procedure

Experiments were conducted between 1830 and 2200 hours. Wind speed and direction were measured nightly using a hand-held anemometer (Heavy weather WS - 2300 or WS – 2310). Wind speed was consistently less than 0 m/s even though experiments were conducted outdoors. Thirty female mosquitoes were placed in the middle chamber of each taxis box and left to acclimatize for 20 minutes. The metal barriers were pulled up and left open for 2 hours, allowing mosquitoes time to respond to the stimulus, after which they were closed. The next morning, mosquitoes were collected from the chambers using mouth aspirators.

5.3.4. Experiment 2: Protective distance of coils against outdoor biting mosquitoes

Semi-field tunnel cage

The semi-field tunnel (SFT) is 100 meters long, 2m wide and 2.5 high.. The walls and roof of the tunnel are screened with fiberglass netting supported by metal frames. A palm-thatch roof approximately 1m above the netting roof protects the tunnel from direct sunlight and rain. The tunnel was operated at temperatures of 24°C - 29°C at night.

Experimental design

Point source experiments

A partially randomized study was conducted inside the SFT. Treatments included 1) control (human alone) and 2) treatment (two 0.03% Transfluthrin coils next to a human). The SFT was divided into two equal compartments, each measuring 30m x 2m x 1.5 m. A plastic sheet between the compartments prevented airflow between them. On the first night of experiments, treatments and two volunteers were randomly allocated to each compartment. This was followed by a pairwise rotation of volunteers and treatments between compartments on consecutive experimental nights. The control was always conducted first in the chosen compartment followed by the treatment in the other compartment after 2 hours on the same night. In the treatment, two 0.03% Transfluthrin coils were placed at a specified distance from the volunteer, hence creating a single source from which the chemical was released (Figure 5.2). This arrangement is referred to as “point source”. The protective distance of coils was evaluated by placing two coils at six different distances from the human at 0.3m, 1m, 5m, 10m, 15m, 20m and 30m. These distances were randomly allocated to each experimental night and each distance was repeated four times.

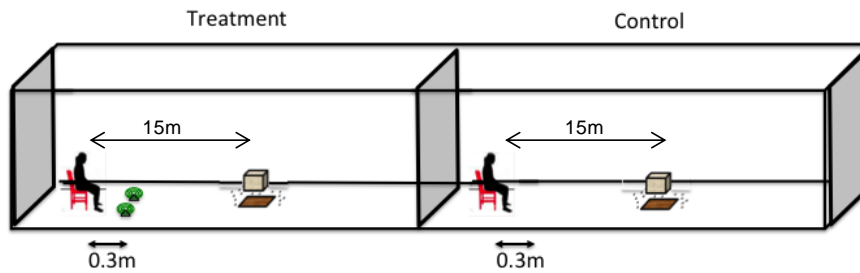


Figure 5.2: Point source experimental set up

Two coils were placed on one side of the human conducting human landing catches. The distance between the coils and the human was changed each day to determine the protective distance. In the control no coils were used. Mosquitoes were released within the tunnel and left to acclimatize for 10 minutes before the human started conducting mosquito catches. The semi-field tunnel measures 100m long, 2m wide and 2.5m high.

Bubble experiments

A partially randomized study was conducted. Treatments included 1) control (human alone) and 2) treatment (two 0.03% Transfluthrin coils next to a human). The same two volunteers from the “point source” experiment also conducted the “bubble” experiment. Experiments were conducted in a 60m long compartment. Unlike the point source, treatments had to be tested on separate days from the control to minimize contamination of the control experiment with residues from burning coils. Treatments were allocated to day one and day two and a volunteer was allocated to each night. Volunteers were switched between nights such that at the end of 4 days both volunteers had been paired with the control and treatment once, which resulted into a four – day block. Six distances (0.3m, 1m, 5m, 10m, 15m, 20m and 30m) were randomly allocated to each four – day block. In this set up, one coil was placed equidistant on the left hand side and another coil on the right hand side of the volunteer at the designated distance creating a “bubble” of chemical around the volunteer (Figure 5.3).

Experiments were started at 1830 hours each evening. One hundred female *An. gambiae* s.s. aged between 3 and 8 days and previously starved for 6 hours were released from cages placed inside the tunnel by a pulley system (Figure 5.3) and operated from outside the tunnel. Mosquitoes were left to acclimatize for 20 minutes after which a volunteer entered the tunnel. Volunteers collected mosquitoes that landed on the bare legs and feet for 2 hours using mouth aspirators. Mosquitoes were kept in labelled paper cups for counting the following

morning. All mosquitoes were kept in the testing room whose temperature was maintained between 28 – 29°C and 70 - 80% relative humidity.

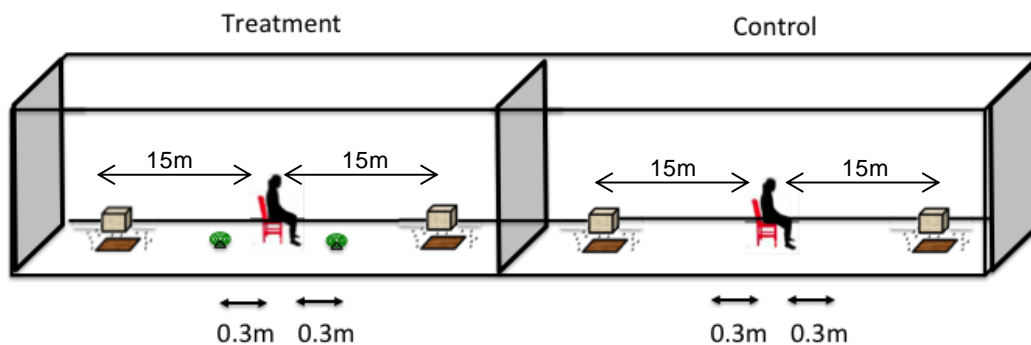


Figure 5.3: Bubble experimental set up

A coil was placed equidistantly on either side of the human. The distance was changed each night to determine the protective distance. Coils were not used in the control. Mosquitoes were released within the tunnel and left to acclimatize for 10 minutes before the human started conducting mosquito catches.

5.3.5. Experiment 3: Resumption to blood feeding of mosquitoes after exposure to coils

Peet Grady chamber tests

Experimental design

A fully randomized study was conducted. Treatments included; 1) a negative control (no coil) 2) blank coil; 3) 0.015% coil; 4) 0.03% coil and 5) 0.045% coil. These treatments were randomly assigned to five days of experiments in a 5 x 5 Latin square design. One hundred female mosquitoes exposed to a treatment were randomly divided into equal batches of 10 mosquitoes per cup. Two cups of mosquitoes were randomly assigned to each blood feeding time regime, namely 15 minutes, 1 hour, 12 hours and 24 hours blood feeding after exposure to coils. Each treatment was repeated five times.

Procedure

The Peet Grady chamber [29] was fitted with a battery operated fan to provide ventilation. One hundred female mosquitoes were placed in 30cm by 30cm netting cages at 1830 hours. A treatment was applied (e.g. a 0.03% Transfluthrin coil was lit) inside the chamber and after 10 minutes, the cage containing mosquitoes was placed inside on a stool. Mosquitoes were exposed to the burning coil for two minutes after which they were transferred to the laboratory and the coil was

extinguished. Mosquitoes were kept in a testing room with temperature maintained between 28°C – 29°C and between 70 - 80% relative humidity. Mosquitoes were gently aspirated and placed into paper cups labelled with the allotted blood feeding time. Pieces of cotton wool soaked in 10% glucose solution were placed on the remaining paper cups. The cotton wool was removed six hours prior to each specific feeding time. After each time interval had elapsed, a human arm was placed above the paper cups and mosquitoes were allowed to feed through the netting for 15 minutes. The number of fed and unfed mosquitoes in each cup was counted and recorded. Experiments with the control were conducted in the same way except that mosquitoes were not exposed to a coil.

Semi-Field Tunnel tests

The experiment included two treatments in the SFT; 1) control (no coil) and 2) treatment (two 0.03% Transfluthrin coils). Treatments were randomly allocated to two days of experiments and one treatment was tested each day. Female mosquitoes were simultaneously exposed to the treatments in the SFT in two different ways; 1) caged mosquitoes and 2) free flying mosquitoes. Experiments were conducted in a 20-meter long SFT lined with white plastic sheets to enable easy location of mosquitoes that were knocked down. Both treatments were repeated four times.

Procedure

Experiments were started at 1830 hours. In the caged mosquitoes set up, 25 female mosquitoes were each placed in four 30 cm by 30cm netting cages. Cages were suspended inside the tunnel one meter above the floor approximately half a meter apart from each other and from two burning 0.03% Transfluthrin coils placed on the floor. In the free flying mosquitoes set up, 100 female mosquitoes were placed in a 30 cm by 30cm netting cage. The cage was placed in the middle of the chamber. A pulley was operated outside the tunnel to release mosquitoes to fly freely inside the tunnel. For both assays, mosquitoes were left in the tunnel for two hours after which caged mosquitoes were removed and free flying mosquitoes were recaptured using mouth aspirators. Knocked down and dead mosquitoes were collected from the floor. All mosquitoes were kept in the testing room whose temperature was maintained between 28°C – 29°C and 70 - 80% relative humidity. Live mosquitoes were placed into paper cups. Two paper cups were allocated to each blood feeding time regime. The time regimes were 1 hour, 12 hours, 18 hours, and 24 hours after mosquitoes had been exposed to burning coils or the control. Mosquitoes were blood fed at the allocated time by placing an arm above the cup for 15 minutes and the number of fed and unfed mosquitoes was recorded. Pieces of cotton wool soaked in 10% glucose solution were placed on paper cups to maintain mosquitoes in between blood feeding. The glucose pads were removed six hours prior to blood feeding.

5.3.6. Protection of participants and ethical approval

The volunteers were recruited on a voluntary basis through written informed consent. The risks and benefits of the study were clearly explained, and they were free to leave at any time during the study. Volunteers were provided with clothing that protected them from the cold temperature at night and were advised to dress in shorts that reached the knees with covered shoes to avoid bites on the feet. They were required not to smoke, take alcohol or use scented soaps and deodorants six hours prior to experiments. The participants were screened for malaria at the beginning of the study and those found with malaria were given Artemisinin Combination Therapy antimalarial drugs and referred to the nearest health centre. Those fit to participate in the study were tested for malaria every two weeks. Adverse events such as respiratory symptoms were monitored. The participants were also compensated for their time and effort. The ethical review boards of Ifakara Health Institute IHI/IRB/No A-019-2007, the National Malaria Research Institute Tanzania (NIMR/HQ/R.8a/Vol.1X/710) and the London School of Hygiene and Tropical Medicine (LSHTM ERB 5552) approved the study.

5.3.7. Statistical analysis

Data was analyzed using the R statistical software version 2.15.0 [30] with significance level of 0.05 for rejecting the null hypothesis. All generalized linear mixed models (GLMMs) were conducted using the lme4 package [31].

Experiment 1: Orientation of mosquitoes in the presence of coils and humans

Activation of mosquitoes to the stimulus

It was assumed that the distribution of mosquitoes in the taxis boxes is a result of movement in response to stimuli. We set the proportion of mosquitoes that were activated by the stimuli equal to the proportion of mosquitoes that left the middle chamber. This was determined by dividing the total number of mosquitoes in the away and towards chamber by the total number of mosquitoes in the taxis boxes including those in the middle chamber. Generalized mixed effects models with binomial error structure and logit link function were used to analyze the behaviour of mosquitoes in taxis boxes. The dependent variable was the proportion of activated mosquitoes. Independent variables included treatment and taxis box code as fixed factors. The taxis boxes were considered as a fixed effect because only two boxes were used. Day was a random factor.

Attraction of mosquitoes to the stimuli

Mosquitoes that were collected from the chamber towards the stimuli were considered to be attracted to the stimulus. Therefore, the proportion of attracted mosquitoes was determined by dividing the number of mosquitoes found in the chamber towards the stimuli by the total number of mosquitoes in the taxis box. Attraction of mosquitoes was analyzed using a GLMM with binomial error structure and logit link function. The dependent variable was the proportion of

attracted mosquitoes. The independent variables were treatment and taxis box code as fixed factors and day as a random factor.

Repellency of mosquitoes by the stimuli

Mosquitoes found in the chamber away from the stimuli were considered to be repelled. This was determined by dividing the number of mosquitoes in the away chamber by total number of mosquitoes in the taxis boxes. Mosquitoes repelled were analyzed using a GLMM with binomial error structure and logit link function. The dependent variable was the proportion of repelled mosquitoes. The independent variables were treatment and taxis box code as fixed factors and day as a random factor.

Experiment 2: Protective distance of coils against outdoor biting mosquitoes

Data from the point source and bubble experiments were analyzed separately. GLMMs were used to determine the proportion of biting mosquitoes at different distances with reference to the control. The dependent variable was the proportion of blood fed mosquitoes while the independent variables included treatment (control and coil), distance and their interaction, which were fixed categorical variables. The day of experiment was included as a random variable. The models were fitted with a binomial error and a logit link function.

Experiment 3: Resumption to blood feeding of mosquitoes after exposure to coils

The data from the Peet Grady, caged and free flying experiments were analyzed separately. GLMMs were fitted with a binomial error and a logit link function. The dependent variable was the proportion of blood fed mosquitoes. Treatment, time regime at which mosquitoes were offered blood and their interaction were set as fixed categorical variables and day of experiment as a random variable.

5.4. Results

5.4.1. Experiment 1: Orientation of mosquitoes in the presence of coils and humans

Activation of mosquitoes

The proportion of activated mosquitoes increased with increasing Transfluthrin dose (Table 5.1). About 82% of the mosquitoes left the middle chamber when 0.045% coils were placed next to the human. The activation of mosquitoes by all the three doses of Transfluthrin was significantly higher compared to the proportion of mosquitoes activated where there was a human alone (Table 5.2). The proportion of activated mosquitoes was lowest (42% – 49%) when there was no Transfluthrin.

Table 5.1: Dose response of mosquitoes to Transfluthrin coils with a human using taxis boxes

| Treatment | Total ^a | Activated ^b | % Activated ^c | 95%CI | Attracted ^d | %Attracted ^e | 95%CI | Repelled ^f | % Repelled ^g | 95%CI |
|----------------|--------------------|------------------------|--------------------------|---------------|------------------------|-------------------------|---------------|-----------------------|-------------------------|---------------|
| No stimulus | 189 | 90 | 48 | [0.40 – 0.55] | 71 | 38 | [0.30 – 0.45] | 19 | 10 | [0.06 – 0.15] |
| Human | 169 | 71 | 42 | [0.35 – 0.50] | 55 | 33 | [0.26 – 0.40] | 16 | 9 | [0.06 – 0.15] |
| Human + blank | 189 | 93 | 49 | [0.42 – 0.57] | 73 | 39 | [0.32 – 0.46] | 20 | 11 | [0.07 – 0.16] |
| Human + 0.015% | 178 | 116 | 65 | [0.58 – 0.72] | 103 | 58 | [0.50 – 0.65] | 13 | 7 | [0.04 – 0.12] |
| Human + 0.030% | 178 | 121 | 68 | [0.61 – 0.75] | 90 | 51 | [0.43 – 0.58] | 31 | 17 | [0.12 – 0.23] |
| Human + 0.045% | 185 | 151 | 82 | [0.75 – 0.87] | 128 | 69 | [0.62 – 0.76] | 23 | 12 | [0.08 – 0.18] |

^a – total number of mosquitoes recovered from all chambers of the taxis boxes; ^b – sum of mosquitoes in the towards and away chamber; ^c – percentage proportion of mosquitoes in the towards and away chamber divided by total mosquitoes in the taxis box; ^d – number of mosquitoes in the towards chamber; ^e – percentage proportion of mosquitoes in the towards chamber divided by total mosquitoes in the taxis box; ^f – number of mosquitoes in the away chamber; ^g – percentage proportion of mosquitoes in the away chamber divided by total number of mosquitoes in the taxis box, CI – Confidence intervals.

Table 5.2: The proportion of activated mosquitoes in taxis boxes placed 1 meter away from different doses of mosquito coils and a human.

| Treatment | Odds ratio | 95% CI | Proportion activated ^a | 95% CI | z Value | p Value |
|--------------------------|------------|----------------|--------------------------------------|---------------|---------|---------|
| Human | 1.00 | [0.57 – 2.18] | 0.42 | [0.30 – 0.54] | - | - |
| Human + blank | 1.34 | [0.87 – 3.27] | 0.49 | [0.32 – 0.66] | 0.824 | 0.410 |
| Human + 0.015% | 2.58 | [1.62 – 6.66] | 0.67 | [0.49 – 0.80] | 2.845 | 0.004 |
| Human + 0.030% | 2.93 | [1.90 – 7.30] | 0.68 | [0.51 – 0.81] | 3.034 | 0.002 |
| Human + 0.045% | 6.13 | [3.95 – 15.92] | 0.82 | [0.69 – 0.91] | 4.988 | 0.001 |
| No stimulus ^b | 1.25 | [0.80 – 3.13] | 0.48 | [0.31 – 0.65] | 0.735 | 0.462 |

^a - Model estimated mean proportions of activated mosquitoes; CI – Confidence intervals; ^b – There was no human or coil, representing movement of mosquitoes in response to nature. The proportion of activated mosquitoes was calculated by dividing the number of mosquitoes collected from the chambers of taxis boxes facing towards and away the treatment by mosquitoes collected from all chambers of the taxis box.

Attraction and repellency of mosquitoes

Approximately half of the mosquitoes were attracted when 0.015% and 0.03% Transfluthrin coils were used (Table 5.1 and Table 5.3). The highest dose of Transfluthrin (0.045%) induced a significantly higher proportion of attracted mosquitoes (69%) relative to the human alone (33%) ($z = 5.160$; $p = 0.001$) (Table 5.3).

The proportion of repelled mosquitoes ranged between 7% and 17% (Table 5.1) and was not significantly different from the human alone (human + blank coil: $z = 0.296$; $p = 0.767$, human + 0.0015%: $z = -0.656$; $p = 0.572$, human + 0.03%: $z = 1.895$; $p = 0.058$, human + 0.045%: $z = 0.789$; $p = 0.430$, human alone: $z = 0.185$; $p = 0.853$). This indicates that the taxis boxes did not detect movement of mosquitoes away from coils and humans.

Table 5.3: The proportion of attracted mosquitoes in taxis boxes placed 1 meter away from different doses of mosquito coils and a human.

| Treatment | Odd ratios | 95% CI | Proportion attracted ^a | 95% CI | z Value | p Value |
|---------------------------------|-------------------|---------------|----------------------------------------------|---------------|----------------|----------------|
| Human | 1.00 | [0.70 – 1.74] | 0.32 | [0.23 – 0.42] | - | - |
| Human + blank | 1.30 | [0.97 – 2.43] | 0.38 | [0.25 – 0.53] | 0.917 | 0.359 |
| Human + 0.015% | 2.85 | [2.08 – 5.41] | 0.58 | [0.43 – 0.72] | 3.639 | 0.001 |
| Human + 0.030% | 2.12 | [1.57 – 3.95] | 0.50 | [0.36 – 0.65] | 2.531 | 0.011 |
| Human + 0.045% | 4.65 | [3.43 – 8.81] | 0.69 | [0.55 – 0.81] | 5.160 | 0.001 |
| No stimulus ^b | 1.25 | [0.92 – 2.34] | 0.38 | [0.25 – 0.52] | 0.822 | 0.411 |

^a - Model estimated proportions of attracted mosquitoes; CI – Confidence intervals; ^b – There was no human or coil, representing movement of mosquitoes in nature. The proportion of attracted mosquitoes was calculated by dividing the number of mosquitoes collected from the chambers of taxis boxes facing towards the treatment by mosquitoes collected from all chambers of the taxis box.

5.4.2. Experiment 2: Protective distance of coils against outdoor biting mosquitoes

Coils placed on one side of the human: ‘point source’.

Smoke from Transfluthrin coils prevented mosquitoes from effectively locating hosts with fewer mosquitoes landing in the presence of coils. Coils were most effective when placed 0.3m away from volunteers. Approximately 20% (95% CI [0.12; 0.31]) of the mosquitoes fed when the coil was 0.3m away compared to 65% (95% CI (0.51; 0.76) when there was no coil ($z = 12.206$; $p < 0.001$) (Table 4). The proportion of feeding mosquitoes also decreased when coils were placed between 1m and 20m (Table 5.4). There was no significant reduction of blood feeding mosquitoes when coils were placed 30m away (Table 5.4).

Coils placed on the left and right side of the human: ‘bubble’

Coils were most effective when they were placed 0.3m away from the human (Table 5.5). Approximately 4% (95% CI [0.01; 0.13]) of the mosquitoes fed when the coil was 0.3m away compared 86% (95% CI [0.66; 0.95] when there was no coil ($z = -5.546$; $p < 0.001$) (Table 5.5). The odds of mosquitoes landing on a human next to a coil increased slightly as the distance between the coils and the human increased (Table 5.5). There was no significant difference in the proportion of landing mosquitoes when coils were placed 30m away (Table 5.5).

Table 5.4: The proportion of mosquitoes that blood feed on humans in the presence of 0.03% Transfluthrin coils placed as a point source at different distances

| Treatment | Distance | Biting /Total | Mean proportion ^a | 95% CI | z Value | p Value | Odds ratio |
|-----------|----------|------------------|---------------------------------|---------------|---------|---------|------------|
| Control | 0.3m | 257/400 | 0.65 | [0.51 - 0.76] | - | - | 1.00 |
| Treatment | | 80/400 | 0.20 | [0.12 - 0.31] | -12.206 | <0.001 | 0.14 |
| Control | 1m | 167/400 | 0.41 | [0.28 - 0.55] | - | - | 1.00 |
| Treatment | | 88/400 | 0.21 | [0.12 - 0.31] | -6.153 | <0.001 | 0.39 |
| Control | 5m | 177/400 | 0.44 | [0.31 - 0.58] | - | - | 1.00 |
| Treatment | | 114/400 | 0.28 | [0.18 - 0.41] | -4.709 | <0.001 | 0.50 |
| Control | 10m | 394/400 | 0.90 | [0.83 - 0.94] | - | - | 1.00 |
| Treatment | | 274/440 | 0.63 | [0.49 - 0.75] | -9.017 | <0.001 | 0.19 |
| Control | 15m | 344/440 | 0.79 | [0.67 - 0.87] | - | - | 1.00 |
| Treatment | | 252/440 | 0.57 | [0.43 - 0.71] | -6.595 | <0.001 | 0.37 |
| Control | 20m | 347/440 | 0.80 | [0.69 - 0.88] | - | - | 1.00 |
| Treatment | | 273/400 | 0.63 | [0.48 - 0.75] | 5.535 | <0.001 | 0.44 |
| Control | 30m | 147/400 | 0.33 | [0.22 - 0.47] | - | - | 1.00 |
| Treatment | | 156/400 | 0.36 | [0.24 - 0.50] | 0.713 | 0.476 | 1.10 |

Legend: ^a – Model estimated mean proportions, CI Confidence intervals

Table 5.5: The proportion of mosquitoes that blood feed on humans in the presence of 0.03% Transfluthrin coils creating a ‘bubble’

| Treatment | Distance | Biting /Total | Mean proportion ^a | 95% CI | z Value | p Value | Odds ratio |
|-----------|----------|------------------|---------------------------------|---------------|---------|---------|------------|
| Control | 0.3m | 80/100 | 0.86 | [0.66 - 0.95] | - | - | 1.00 |
| Treatment | | 4/100 | 0.04 | [0.01 - 0.13] | -5.546 | <0.001 | 0.01 |
| Control | 1m | 259/600 | 0.43 | [0.36 - 0.51] | - | - | 1.00 |
| Treatment | | 12/600 | 0.02 | [0.01 - 0.04] | -11.950 | <0.001 | 0.02 |
| Control | 5m | 331/600 | 0.41 | [0.35 - 0.48] | - | - | 1.00 |
| Treatment | | 5/800 | 0.01 | [0.00 - 0.01] | -10.580 | <0.001 | 0.009 |
| Control | 10m | 216/600 | 0.35 | [0.29 - 0.43] | - | - | 1.00 |
| Treatment | | 8/600 | 0.01 | [0.01 - 0.03] | -10.210 | <0.001 | 0.02 |
| Control | 15m | 83/100 | 0.84 | [0.63 - 0.94] | - | - | 1.00 |
| Treatment | | 39/100 | 0.37 | [0.17 - 0.63] | -2.808 | 0.005 | 0.13 |
| Control | 20m | 70/100 | 0.71 | [0.46 - 0.87] | - | - | 1.00 |
| Treatment | | 37/100 | 0.37 | [0.17 - 0.62] | -1.891 | 0.060 | 0.25 |
| Control | 30m | 90/100 | 0.92 | [0.77 - 0.97] | - | - | 1.00 |
| Treatment | | 78/100 | 0.79 | [0.56 - 0.92] | -1.353 | 0.176 | 0.39 |

5.4.3. Experiment 3: Resumption to blood feeding of mosquitoes after exposure to coils

Experiments in the Peet Grady chamber

The proportion of fed mosquitoes was lowest at 12% (95% CI [0.06; 0.22]), ($z = -5.301$; $p < 0.001$) 15 minutes after exposure to 0.03% Transfluthrin coils. The presence of smoke without the insecticide (blank coil) significantly inhibited feeding after 15 minutes (Table 5.6) but the proportion of mosquitoes inhibited from feeding was lower than when Transfluthrin coils were used. The effect of Transfluthrin coils demonstrated a dose response relationship although increasing the dose beyond 0.03% had little effect (Figure 5.4).

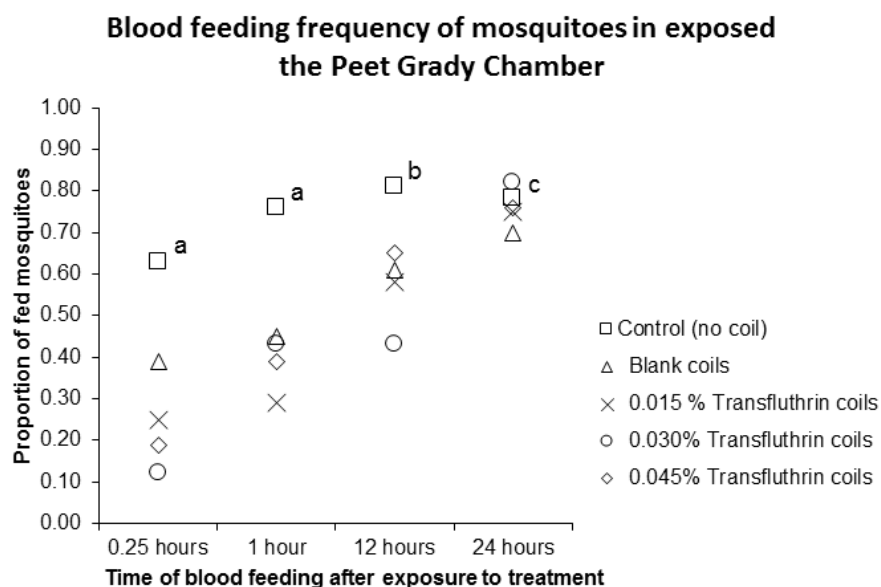


Figure 5.4: The effect of Transfluthrin coils on blood feeding behaviour of mosquitoes.

Mosquitoes were exposed to different doses of Transfluthrin coils inside a Peet Grady chamber and later offered blood meals at different time intervals. The proportion of blood fed mosquitoes was significantly lower than the control in all treatments after 25 minutes (^a) and 1 hour (^a). At 12 hours only 0.03% Transfluthrin coils significantly (^b) reduced feeding compared to the control while after 24 hours there was no significant difference between all treatments and controls (^c).

Exposure to burning coils also influenced subsequent blood feeding. The proportion of mosquitoes that took blood up to 12 hours after exposure to 0.03% and 0.045% Transfluthrin coils were significantly lower compared to the control (Table 5.6). In addition, the propensity of mosquitoes to feed increased gradually with time irrespective of whether they were exposed to Transfluthrin coils or not. Results indicate that at some point between 12 and 24 hours, there was no difference in the proportion of fed mosquitoes between the control and coils

(Table 5.6), showing that mosquitoes resume normal feeding one day after indoor exposure to Transfluthrin coils.

Table 5.6: The proportion of mosquitoes that blood fed at different time intervals following exposure to different doses of Transfluthrin coils inside a Peet Grady chamber

| Treatment | Time (Hours) | Fed /Total | Mean proportion ^a | 95% CI | z Value | p Value | Odds ratio |
|------------------|-------------------------------|-----------------------------|-----------------------------------------------|---------------|----------------|----------------|-----------------------------|
| Control | | 61/98 | 0.63 | [0.49 - 0.76] | - | - | 1.00 |
| Blank | | 42/100 | 0.39 | [0.27 - 0.53] | -2.409 | 0.174 | 0.53 |
| 0.015% | 0.25 | 23/99 | 0.25 | [0.15 - 0.38] | -3.815 | 0.002 | 0.18 |
| 0.030% | | 12/99 | 0.12 | [0.06 - 0.22] | -5.301 | <0.001 | 0.08 |
| 0.045% | | 20/100 | 0.19 | [0.11 - 0.31] | -4.393 | <0.001 | 0.08 |
| Control | | 74/100 | 0.76 | [0.62 - 0.85] | - | - | 1.00 |
| Blank | | 47/98 | 0.45 | [0.32 - 0.59] | -3.115 | 0.025 | 0.32 |
| 0.015% | 1 | 26/98 | 0.29 | [0.18 - 0.42] | -4.674 | <0.001 | 0.13 |
| 0.03% | | 43/100 | 0.43 | [0.29 - 0.54] | -3.267 | 0.015 | 0.13 |
| 0.045% | | 39/99 | 0.39 | [0.26 - 0.53] | -3.610 | 0.005 | 0.23 |
| Control | | 78/98 | 0.81 | [0.69 - 0.89] | - | - | 1.00 |
| Blank | | 58/93 | 0.61 | [0.47 - 0.73] | -2.254 | 0.245 | 0.42 |
| 0.015% | 12 | 55/100 | 0.58 | [0.45 - 0.71] | -2.544 | 0.126 | 0.31 |
| 0.030% | | 41/94 | 0.43 | [0.30 - 0.58] | -3.810 | 0.002 | 0.20 |
| 0.045% | | 60/93 | 0.65 | [0.50 - 0.77] | -1.842 | 0.516 | 0.47 |
| Control | | 71/93 | 0.78 | [0.65 - 0.87] | - | - | 1.00 |
| Blank | | 66/93 | 0.70 | [0.57 - 0.81] | -0.915 | 0.992 | 0.76 |
| 0.015% | 24 | 69/96 | 0.75 | [0.63 - 0.84] | -0.379 | 1.000 | 0.79 |
| 0.03% | | 71/88 | 0.82 | [0.69 - 0.90] | 0.443 | 1.000 | 1.29 |
| 0.045% | | 64/85 | 0.76 | [0.62 - 0.86] | -0.273 | 1.000 | 0.94 |

Legend: ^a – Model estimated mean proportions, CI Confidence intervals

Experiments in the Semi-Field Tunnel

Two Transfluthrin coils (0.03%) did not influence the feeding behavior of free flying mosquitoes exposed outdoors in the netting tunnel. The proportion of mosquitoes that fed after exposure to coils was not significantly different from the control ($z = -0.943$; $p = 0.346$); around 53% (42/76) (95% CI [0.43; 0.67]) blood fed after 1 hour after exposure to Transfluthrin compared to 61% (48/80) (95% CI [0.48; 0.71]) in the control. More than three quarters of the mosquitoes had fed after 12 hours and subsequent time feeding intervals. There was no significant difference between the proportion of fed mosquitoes in the control and the treatment at subsequent feeding times (12 hours: $z = 0.526$; $p = 0.599$, 18 hours: $z = -0.169$; $p = 0.866$, 24 hours: $z = -0.098$; $p = 0.922$).

There was a slight impact on the feeding behaviour of caged mosquitoes. After 1 hour, 56% (35/62) (95% CI [0.43; 0.69]) mosquitoes exposed to Transfluthrin fed compared to 79% (61/77) (95% CI [0.69; 0.88]) in the control ($z = -2.937$; $p = 0.003$) and after 18 hours 84% (59/70) (95% CI [0.74; 0.92]) mosquitoes had blood fed compared to 72% (39/54) (95% CI [0.58; 0.84]) in the treatment ($z = -2.445$; $p = 0.015$). However, more than three quarters of the mosquitoes fed after 12 hours and 24 hours and this was not significantly different between the control and the treatment (12 hours: $z = -1.341$; $p = 0.180$, 24 hours: $z = -0.006$; $p = 0.996$).

5.5. Discussion

This study highlights challenges in the measurement of mosquito responses to different stimuli whilst outdoors. Mosquito activity such as orientation towards hosts, oviposition and resting sites are largely influenced by external stimuli such as atmospheric carbon dioxide, light sources, humans, animals and wind. In the taxis box experiment, mosquitoes moved between chambers even when there was no coil or human (no stimuli). This shows that mosquitoes may have been responding to external stimuli within the taxis boxes, making it quite difficult to discern between mosquito responses to experimental or external stimuli. Despite these challenges, taxis boxes have been used to successfully demonstrate orientation of malaria mosquitoes towards attractive stimuli outdoors in field conditions [7]. In the current study, taxis boxes were used to determine attraction and repellency of airborne pyrethroids.

Here, Transfluthrin coils placed next to a human increased movement of mosquitoes within the taxis boxes. A higher proportion of mosquitoes left the middle chamber and flew towards the human. The presence of transfluthrin did not hinder movement of mosquitoes towards the human, hence they did not inhibit attraction. In fact, an increase in the dose of transfluthrin increased activation and attraction of mosquitoes. This behaviour has been previously reported in other study as excito-repellency [25]. Pyrethroid coils are thought to cause excitation and increased activity of mosquitoes. This may explain high activation observed in the current study. A low proportion of mosquitoes (7% and 17%) moved away from the human even when there was a coil. This indicates that Transfluthrin coils

did not induce movement away from the coil. However, there was no difference between the proportion of mosquitoes that moved away from the human alone and the human and coil. This questions the suitability of taxis boxes to accurately measure repellency outdoors where there are a lot of competing stimuli, highlighting the need for better methods to test repellency in the field.

Mosquitoes seen to fly towards humans even in the presence of coils indicated that coils did not inhibit attraction to the human. It is possible that coils actually work at close range resulting in other responses such as bite prevention [24].

Similar observations are reported elsewhere describing the effect of metofluthrin emanators and pyrethroid coils [24,32,33]. Catnip and 1-methylpiperazine acts at short distances to prevent mosquitoes from landing and biting humans but do not prevent attraction to attractive stimuli [34,35]. This study reinforces the fact that airborne pyrethroids do not prevent attraction of mosquitoes to their hosts but likely interfere with the mosquito feeding process at the last stages after attraction to the host and prevent blood feeding. Other studies show that airborne pyrethroids exert multiple effects on a range of odorant receptors (ORs) and gustatory receptors located on antennae and feeding appendages of mosquitoes. They block, inhibit, or induce a number of different responses and scramble the host seeking process [22,36,37].

In this experimental design, mosquitoes were presented with conflicting stimuli: attraction to host odours versus the insecticide. A previous study showed that in such a case the need to feed can overpower the effect of the insecticide, hence the mosquito is still attracted to the host but is prevented from feeding [38]. This is

evident with the use of insecticide treated bed nets, where mosquitoes are attracted to humans and attempt to feed through treated nets but then become irritated and move away without feeding [38]. This information is useful in the development of “push – pull” strategies that include compounds thought to chase mosquitoes from humans and attract them to odour-baited traps [11,34].

Coils used as a “point source” reduced bites by almost half when coils were placed 0.3m away from the human and were effective even when the human was 20m away from coils (Table 5.4). Interestingly the “bubble” was highly effective providing approximately 80% protection against bites when coils were 0.3m away from the human (Table 5.5). Hence coils were more effective when used as a “bubble” rather than the “point source”. This highlights the need to consider presentation of the source of the active ingredient as a bubble around humans in order to achieve maximum efficacy. These results show the spatial activity and efficacy of volatile pyrethroids against mosquito bites. Efficacy of coils outdoors indicates that volatile pyrethroids may be an appropriate tool against outdoor biting mosquitoes and may be used outdoors in bars, restaurants, backyards or verandas especially when multiple sources of repellent are used to ensure saturation of the space with active ingredient.

Previous studies indicate that mosquitoes inhibited by topical repellents from blood feeding are diverted to neighbouring people who are not protected [39]. This may not be the case with volatile insecticides such as mosquito coils. This study shows that coils prevented bites when they were placed as far as 15m away (Table 5.4 and 5.5.), thus they provided area wide protection and hence are likely

extend protection to the non users at a particular distance from the source and reduce risk of diversion of mosquitoes. A study testing this hypothesis is currently being analyzed (Maia *pers. comm.*).

In addition to the spatial mode of action of coils, this study also shows that they offer temporal protection. In a closed laboratory setting (Peet Grady chamber), mosquitoes did not resume normal blood feed behaviour up to 12 hours after they had been exposed to coils. We suggest that in addition to a spatial bubble, prolonged feeding inhibition may also protect non-users of coils to a certain extent, which would also reduce the risk of diversion. Similar results were reported in a study where the time of activation and flight of *Cx. quinquefasciatus*, *An. albimanus* and *Stegomyia aegypti* mosquitoes was reduced significantly 24 hours after they had been exposed to sublethal doses of Deltamethrin and Permethrin [40]. In the current study, mosquitoes resumed normal feeding after 24 hours. If mosquitoes miss one feeding opportunity due to exposure to coils, this is likely to prolong the gonotrophic cycle and may change the vectorial capacity of the mosquitoes [41].

However, when free-flying mosquitoes were exposed to coils under outdoor conditions in the SFT, there was no effect. This may be attributed to limited ventilation in the Peet Grady chambers resulting in reduced airflow accompanied by increased insecticide particles per area. This enabled mosquitoes to contact insecticides more easily, resulting in the large effect on blood feeding inhibition in mosquitoes exposed in the chambers. The effect of coils in the SFT was less pronounced probably due to the large surface area of the facility as well as natural

airflow within the tunnel. It is hypothesized that sparse distribution of insecticide particles within the tunnel due to high airflow resulted in low concentration of insecticide particles. Therefore, mosquitoes did not contact sufficient insecticides in the SFT. It should be noted that coils used under outdoor conditions contained the standard dose of Transfluthrin (0.03%) meant for indoor use. It is therefore necessary to explore the effect of increasing the dose for products that are intended for outdoor use, in particular by advising users to put several coils around the area that they are occupying to create the “bubble effect”. In addition, there is need to determine the No observed effect level (NOEL) of airborne chemicals whilst in use outdoors.

The human biting rate of mosquitoes is one of the most important parameters that influences malaria transmission [3]. Hence, chemicals that interfere with feeding behaviour of mosquitoes or prevent feeding altogether are likely to reduce transmission. This study emphasizes the importance of reduced blood feeding as the main indicator for efficacy of airborne pyrethroids used against outdoor biting mosquitoes.

5.6. Conclusion

This study indicates that coils do not prevent attraction to the human, but mainly prevent blood feeding. It is possible that pyrethroid based coils, specifically Transfluthrin, target gustatory receptors involved in feeding rather than olfactory receptors. It is essential to conduct further studies to determine target sites of pyrethroid - based airborne particles in mosquitoes. This study provides critical information necessary for the development of target product profiles of spatial repellent products that can be used to complement existing mainstream malaria vector control tools.

Increased reports of outdoor biting and resting mosquitoes in endemic areas [42,43] indicate that mainstream malaria control tools that target indoor biting and resting mosquitoes (LLINs and IRS) may not be sufficient to eliminate malaria especially when transmission occurs outdoors [44]. This study demonstrates the potential benefit of airborne pyrethroids for use against outdoor biting mosquitoes by reducing the outdoor man-biting rate, an important parameter of malaria transmission. It is worthwhile to conduct large scale clinical studies with entomological correlates of mosquito human-landing also observed to determine whether outdoor use of airborne insecticides in addition to the use of LLINs translates into additional protection from malaria, therefore complementing existing tools used against indoor biting and resting mosquitoes.

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CHAPTER SIX

6 Spatial repellency of transfluthrin-treated hessian strips against laboratory-reared *Anopheles arabiensis* mosquitoes in a semi-field tunnel cage

6.1. Abstract

Background

Vapour phase spatial repellents deter mosquitoes from attacking one or more humans in a protected space. Simulation models indicate that high coverage of spatial repellents can enhance the impact of Long Lasting Insecticidal Nets (LLINs) and indoor residual spraying (IRS) where mosquito vectors commonly bite humans outdoors. Here we report a preliminary evaluation of an effective, user-friendly prototype product for delivering spatial repellents to protect against malaria vector mosquitoes.

Findings

Protective efficacy of a 4.0×0.3 m strip of hessian sacking treated with 10 ml of transfluthrin was evaluated in a $60 \text{ m} \times 2 \text{ m} \times 2.5 \text{ m}$ netting tunnel with malaria-free insectary-reared *Anopheles arabiensis* Patton mosquitoes. Personal protection, in terms of proportional reduction of exposure to bites, was measured by comparing human landing catches of volunteers with treated and untreated

strips. A freshly treated hessian strip reduced mosquito attack rate on human volunteers by > 99% and consistently conferred > 90% protective efficacy for a period of 6 months. Over the entire study period, only 22 out of 1400 released mosquitoes bit volunteers using the treated sacking strip while 894 out of 1400 mosquitoes released into cages containing volunteers using an untreated strip fed upon them.

Conclusion

Locally available natural fibres may be promising absorbent substrates for delivering spatial repellents, such as transfluthrin, to protect against mosquitoes in tropical settings. However, these observations relate to a single prototype specimen of this particular device, therefore, much more detailed, well replicated studies are essential to establish long-term efficacy, effectiveness, practicability and affordability.

Keywords: Outdoor mosquito control; Spatial repellency; Hessian strips

6.2. Findings

Long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have successfully reduced malaria in many endemic regions of Africa [1-4]. These measures have successfully reduced malaria vectors, which predominantly feed upon humans (anthropophagic) and rest (endophilic) and feed (endophagic) indoors [5-11]. Despite impressive successes, these tools are less effective against exophagic, and exophilic mosquito vectors [12, 13]. It is therefore critical to find

new tools that would protect people whilst outdoors.

Recently developed mathematical models suggest that highly efficacious spatial repellents are likely to be effective when used outdoors in areas where transmission commonly occurs outside of houses [14] or is mediated by mosquitoes which primarily feed upon animals (Kiware et al, Unpublished). Examples of spatial repellent products include mosquito coils and vaporizer mats [15]. Kerosene lamps containing transfluthrin and vegetable oil is a cheap and effective means of dispensing repellents, use of which is well matched to the times and locations of peak human activity [16]. These delivery formats require frequent replacement of the active ingredient and external sources of energy such as combustion or electricity.

Passive methods of delivering spatial repellents without external energy input are highly desirable for impoverished populations in developing countries. Existing products typically consist of paper or plastic strips impregnated with fluorinated pyrethroids, such as metofluthrin or transfluthrin, and have exhibited high efficacy of protection against mosquito bites in some parts of Southeast Asia [17, 18]. These pyrethroids are less polar and highly volatile than conventional pyrethroids and therefore evaporate at room temperature without the need for any external source of energy [19]. Such strips can produce vapour for 18 weeks, during which time it repels mosquitoes or prevents them from feeding on humans [18, 19]. Interestingly, the level of repellency achieved by treated paper strips has been shown to be more short lived than plastic strips treated in exactly the same manner, demonstrating how different substrates can affect the duration of efficacy

exhibited by a given active ingredient [19].

Natural fibres are readily available and affordable in all tropical countries. Initial assessments to compare the physical properties of hessian sacking materials, commonly used for storing and transporting goods in Tanzania, indicated that it had far greater absorbent capacity than commonly available alternatives. The hessian fabric used in this study is made from fine sisal fibres woven together. The fabric is imported from India and is used to make cereal storage bags.

We evaluated the spatial repellency of a hessian sacking strip treated with transfluthrin, in terms of its ability to prevent attack by vectors of malaria in Africa when used outdoors.

Hessian strips 4 m long and 30 cm wide were impregnated with 10 ml technical grade transfluthrin (SC Johnson Home Hygiene Products). A volume of 10 ml of transfluthrin was mixed with 90 ml Axion[®] liquid detergent (Orbit Chemical Industries Ltd, Nairobi and Colgate-Palmolive East Africa Ltd) to enable its solubility in 400 ml of water. The strips were dipped in the mixture in a plastic basin and suspended indoors at ambient temperature where they were left overnight to dry. A negative control was treated exactly the same way using the mixture of detergent and water only, without any transfluthrin active ingredient.

Experiments were conducted in a screened tunnel measuring 60 m long, 2 m wide and 2.5 m high at the Ifakara Health Institute (IHI) facility in Ifakara, Morogoro, United Republic of Tanzania. The tunnel was divided into three equal-sized

experimental units (A, B and C) separated by plastic sheets. Each unit was 20 m long (Moore *et al.* unpublished).

We conducted tests with *Anopheles arabiensis* mosquitoes previously collected from Sakamaganga village, Kilombero valley, South East of the United republic of Tanzania. The mosquitoes were reared in an insectary built within the IHI semi-field system [20]. The temperature in the insectary was between 28 - 29°C and 70-80% relative humidity. Mosquito larvae were fed on tetramin fish food and adults were given 10% glucose solution and blood meals. Nulliparous female, insectary-reared, 2 to 6 day old mosquitoes that had never had a blood meal were used.

Personal protection in terms of the proportion of reduction in mosquitoes attacking volunteers was measured by comparing the number of mosquitoes that landed upon a volunteer with a treated sacking strip and the one who had an untreated strip. Experiments were conducted in units A and C while unit B was used as a buffer zone with no experiments between these two experimental units to minimize the risk that the transfluthrin-treated sacking in one unit would affect mosquitoes in the unit containing the negative control.

Each strip was suspended 1 m above the ground in the middle of each unit on a square frame of 4 wooden poles 1 meter apart, thus creating approximately 1 m² sitting space (Figure 1). Treated and untreated strips were randomly assigned to the units on the first night of every round of 4 nights of experimentation, they were exchanged between units on the third day, and remained in that arrangement for the fourth day. A cage containing 25 mosquitoes was placed at each of the two

opposite ends of each unit so that, at the start of the experiment, a total of 50 mosquitoes were released in each unit. Mosquitoes were released at 1900 hours by pulling strings attached to mosquito netting cages placed on each side of the volunteer. Mosquitoes were recaptured by human landing catches simultaneously in both units for 2 hours each night. The two male participants involved in the study were randomly assigned to the experimental units on the first night using the lottery method. They exchanged positions on the second night. On the third night volunteers were randomly assigned to the units again and exchanged positions on the fourth night. Each round of rotation of volunteers and strips between experimental units was completed in 4 nights. One round of experimentation was repeated once every month to check for residual activity of transfluthrin on the hessian strips. The strips were kept in separate plastic basins and stored uncovered at ambient room temperature indoors.



Figure 6.1: Transfluthrin hessian strip. The hessian strip is made from fine sisal fibre woven together to make sacking fabric. The strip is 4×0.3 m long. It is treated with transfluthrin. The strip is suspended on 4 wooden poles making approximately 1 m^2 area surrounding the human participant conducting mosquito catches.

This study was approved by The National Institute of Medical Research (NIMR/HQ/R.8 C/VOL.1/100). Participants signed a written informed consent form before commencing the study.

The freshly treated sisal strip provided > 99% protective efficacy against mosquitoes: In the first round of assays only 1 mosquito out of 200 that were released was recovered by the volunteer in the experimental unit with a treated strip, while 148 out of 200 released mosquitoes were recovered in the unit with an untreated control. The treated strip continued to consistently confer > 99% protective efficacy for a period of 6 months and all assay rounds, except one during the fourth month, indicated approximately 91% protective efficacy. Over the entire study period only 22 out of 1400 mosquitoes released into the experimental unit with the treated sacking strip were recovered by the protected human catcher. In stark contrast, 894 out of 1400 released mosquitoes bit the volunteers using an untreated sacking strip (Figure 6. 2).

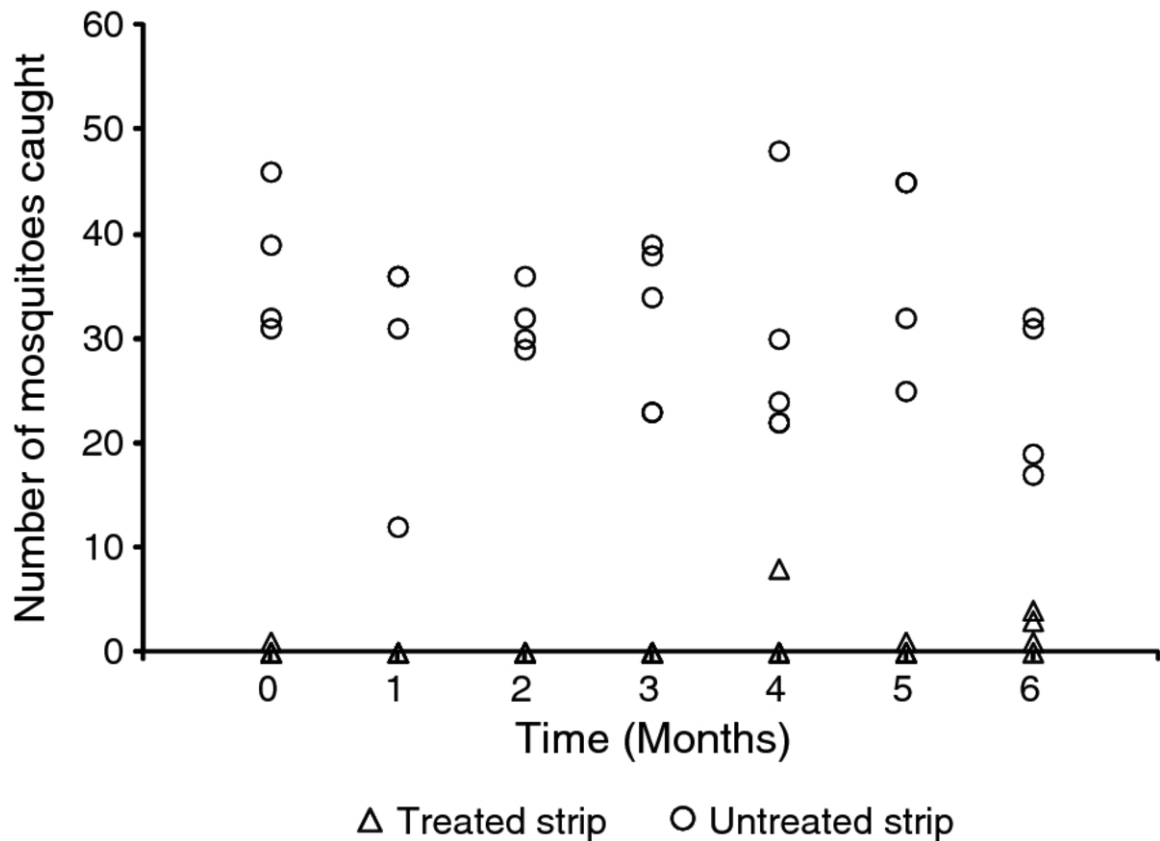


Figure 6.2: The number of mosquitoes recovered by human landing catches with transfluthrin and untreated strips. A graph comparing the number of mosquitoes recovered by human landing catches during rounds of experiments with transfluthrin treated and untreated strips during six months. The graph indicates a reduction in the number of bites occurring on a human participant who had a treated strip compared to one with an untreated strip. Each data point represents a single release of mosquitoes in a single experimental unit with either a treated or untreated strip of hessian sacking.

While a generalized linear mixed model with a Poisson distribution indicated a clear effect of the treatment status of the hessian strip ($P < 0.001$), there was no apparent difference between the participants in terms of their attractiveness to mosquitoes ($P = 0.208$), but the experimental units were significantly different ($P = 0.027$). The latter effect could be explained by external factors such as light from the nearby security lights shining through one of the units.

Such a prototype conferring such high apparent protective efficacy against outdoor-biting *Anopheles* mosquitoes may well be useful for preventing malaria transmission that mostly occurs outdoors. Our results indicate that hessian sacking substrates may be an efficient means for delivering transfluthrin vapour into an occupied space to protect humans against mosquito bites. Hessian and other natural fibres can be affordably produced in tropical countries, even locally within afflicted communities themselves, thus reducing potential costs of transportation and importation because only the active ingredient needs to be manufactured in bulk by specialist chemical manufacturers.

Hessian fibres are a versatile fabric that can be readily woven into a variety of practical formats such as treated wall hangings, door mats or curtains. It might even be possible to weave it into items that can be worn on the body, such as wrist bands, head bands or anklets, so long as the absorbent fibre can be packaged within porous, untreated coating materials that preclude human dermal exposure to the active ingredient.

These preliminary results demonstrated efficacy of transfluthrin strips against mosquitoes under the near-natural conditions of an outdoor semi-field system. However, these observations relate to a single, un-replicated prototype specimen of this particular device [21] so more intensive, well replicated studies in both semi-field systems and full field

settings will be required in order to establish these results and characterize the properties of such devices. In particular, it would be important to conduct experiments in which the control and treatment are exposed to mosquitoes alongside each other at a range of proximities within a single semi-field chamber or in full field settings.

The long-term efficacy of the prototype will need to be evaluated at frequent time intervals after formulation and initiation of routine, representative use in target communities. Also, the relationship between protective efficacy and distance from the product will need to be assessed. In particular, the possibility that vapour-phase repellents which prevent mosquitoes from feeding on humans without killing them might pose a risk to nearby non-users by diverting mosquitoes to them [22, 23], as is known to occur when using some topical repellents [24] will need to be investigated.

When considering use of spatial repellents, it is necessary to take into account how these can be used with existing tools such as LLINs and IRS in order to complement, rather than reduce, their efficacy [14, 22, 23, 25]. Recently developed models indicate that insecticides which deter mosquitoes from entering houses may undermine the community-level impact upon malaria transmission by the contact toxicity of less volatile conventional pyrethroids applied in the form of LLINs and IRS [14, 25]. This is because mosquitoes deterred by sub-lethal doses of an insecticide are prevented from making contact with toxic doses on treated surfaces and are therefore not killed directly. For settings where malaria transmission is dominated, or has historically been dominated, by vectors that typically feed indoors upon humans, it will therefore be essential to assess the mode of action, and community-level impact upon transmission, of products relying

upon vapour phase active ingredients when applied both indoors and outdoors to ensure that they complement rather than attenuate the impact of existing front-line LLIN and IRS technologies.

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CHAPTER SEVEN

7 General discussion

7.1. The mode of action of spatial repellents against mosquitoes

Rapid toxicity of neuro-toxic insecticides is considered the main outcome indicator of efficacy of mosquito control tools such as LLINs and IRS, while sub-lethal effects of insecticides that include spatial repellency, irritancy and reduced human biting rate are considered secondary attributes, and perhaps negative effects of insecticides because they do not result in a directly measurable death of that insect. Several studies have shown that indoor spraying with DDT led to the reduction of malaria transmission through such sublethal effects [1]. More importantly, DDT functions first as a spatial repellent and then a feeding inhibitor [2] while toxicity comes into action slowly and later after repellency [3, 4].

Despite several studies conducted on the topic of spatial repellency, the mode of action and the mechanisms driving the range of behaviours exhibited by mosquitoes are still under debate by the vector-control community. A repellent was described as a chemical that caused the responder to move away from the source of the chemical [5]. More recently the terms irritancy and spatial repellency have been used to distinguish between directed movement away after physical contact with the stimulus and oriented movement away after contact with odour or airborne stimulus, respectively [4]. Miller *et al* suggest

that use of these terms may not be appropriate especially where directional movement is not proven [6]. They suggest reverting to Dethier *et al* where repellency is described with regards to the mechanisms involved. Repellency is described by two terms i.e. taxis – immediate directional avoiding reaction, and orthokinesis – irritating effect, causing insects to increase activity resulting in decreased number on the surface [5, 6]. The term excito-repellency has also been used by some authors to refer to the hyper - locomotor activity observed when some insects contact insecticides [7, 8]. In this thesis spatial repellency generally refers to a range of behaviours exhibited by insects after exposure to airborne chemicals and resulting in reduced human-vector contact. It was hypothesized that airborne insecticides 1) induce mosquitoes to move away from them, 2) interfere with host detection and blood feeding behaviour of mosquitoes and also 3) prevent mosquitoes from flying [9]; and the overall goal of this thesis was to determine the range of mosquito behaviours exhibited by airborne pyrethroids that minimize human - vector contact and consequently reduce malaria transmission by the major Afro-Tropical vectors *An. gambiae s.s.* and *An. arabiensis*.

7.1.1. Irritancy and excito-repellency

One notable feature of this study was that the rate at which mosquitoes left huts was measured throughout the night (excito-repellency and irritancy) [10]. More mosquitoes left huts earlier than normal where both Transfluthrin and Metofluthrin coils and DDT were used. In the semi-field studies reported in Chapter 4, almost half of the mosquitoes (56%, 55% and 48%) prematurely left huts that had Transfluthrin coils, Metofluthrin

coils, and DDT, respectively [10]. High irritancy (82% and 87%) by pyrethrum coils on *An. gambiae s.l.* mosquitoes has been reported [11] while another study indicated 14% irritancy of *Aedes aegypti* by DDT [4]. There are several possible explanations as to why mosquitoes rapidly leave houses upon contact with DDT and airborne pyrethroids. The first one is that mosquitoes contact airborne insecticides and they are irritated or excited (orthokinesis) [5] hence they move around faster than usual [2, 12]. This mechanism may be as a result of the action of DDT and pyrethroids on the voltage-gated sodium channel of insect nerve cells resulting in restlessness, un-coordination and hyperactivity of the insects [13]. It is not clear how this rapid locomotion of mosquitoes causes them to leave houses. Kennedy suggests that mosquitoes are inclined to move towards light and that is why they leave through open spaces in a house [14]. Several experimental hut studies concur with this explanation as demonstrated by exit of mosquitoes through eave gaps [15, 16]. The second explanation may be that low concentrations of insecticides induce loss of response to host cues [17] and prevents mosquitoes from feeding and therefore mosquitoes leave huts in search of other blood sources. Insecticides that cause mosquitoes to leave treated houses before or after they have fed are likely to attenuate efficacy of interventions such as IRS that rely on resting behaviour of mosquitoes. Irritancy and excito-repellency are likely to prevent mosquitoes from contacting lethal insecticides and thus reduce mortality of mosquitoes. Despite this widespread notion, it is also believed that irritancy by DDT led to tremendous success in malaria control [3, 18]. This may be explained by reduced human biting rate and thus reduced malaria transmission. There is need to conduct further studies to determine whether spatial irritancy by spatial repellents (SR) is likely to increase transmission.

7.1.2. Directional taxis and feeding inhibition

In the semi-field experiments in Chapter 5, airborne pyrethroids produced by burning coils did not induce taxis (directional movement away from coils) of mosquitoes.

Interestingly, the taxis boxes results were congruent with experimental hut results [10] and indicated activation of mosquitoes and therefore excito-repellency as the main mode of action of the coils. Increasing the dose of Transfluthrin to 0.045% increased the activity of mosquitoes, implying a dose response relationship. More mosquitoes were activated to move towards the human especially in the presence of coils. This shows that airborne pyrethroids do not hinder attraction of mosquitoes to humans at short distance away from them.

These results are congruent with other studies of airborne pyrethroids. For example in the presence of metofluthrin dispensed by emanators, mosquitoes detected host odours and flew upwind towards the host but they were prevented from biting [19]. Further studies with pyrethroid coils also indicate that coils did not induce repellency but “interfered” with host seeking and prevented feeding [20] and (Chapter 5). This behaviour has been observed with Permethrin treated bed nets. Mosquitoes stayed far much longer on treated bed nets because of the desire to blood feed on humans protected by the nets despite the irritating effect of pyrethroids. Similarly the pyrethroids did not inhibit attraction to humans [21]. It should be noted that chemicals other than pyrethroids such as DEET have been shown to inhibit attraction of mosquitoes to humans [22]. Despite the controversies surrounding the mode of action of DEET, evidence suggests that DEET affects feeding

behaviour of *Drosophila* through the activation of gustatory receptor neuron and hence induces avoidance and suppresses feeding [23]. This might explain the effect of coils on the feeding behaviour of mosquitoes in the Peet Grady chamber and in the SFS. Other studies indicate that DEET inhibits positive olfactory responses to attractive compounds such as Lactic acid and octan-3-ol [22, 24] or acts as a “confusant” through direct modulation of olfactory receptor activity and interferes with behavioral responses such as attraction of mosquitoes to human odours [25, 26]. A comprehensive study on the effect of insect repellents of different chemical structures and repellent pyrethroids on odorant receptors in mosquitoes implies that all repellents modulate the function of odorant receptors. This is either through inhibiting odorant-evoked currents mediated by odorant receptors or through responses elicited in the absence of odours [27, 28]. These studies elucidate mechanisms underlying the response of mosquitoes to repellent compounds. This information is useful in the development of better repellents. For instance pyrethroid repellents act on olfactory receptors rather than target the sodium-gated channels are potential chemicals for spatial repellent products because low amounts of repellent may be needed to achieve high efficacy and this is likely to slow down the development of resistance.

7.1.3. Feeding inhibition

Exposure of mosquitoes to airborne Transfluthrin in the Peet Grady chamber seemed to “jam” the mosquito feeding system for 12 hours when mosquitoes were unable to blood feed (Chapter 5). The prolonged feeding inhibition status is an extremely important

finding because it is likely to also protect non-users of SR by reducing diversion of unfed mosquitoes to unprotected individuals. Similar results have been reported in a study where the time of activation and flight of *Cx. quinquefasciatus*, *An. albimanus* and *Stegomyia aegypti* mosquitoes was reduced significantly for 24 hours after exposure to sublethal doses of Deltamethrin and Permethrin [29]. In the current study, mosquitoes resumed normal feeding after 24 hours. It is hypothesized that if mosquitoes miss one feeding opportunity due to exposure to SR, they are likely to continue host seeking and therefore this may prolong the gonotrophic cycle and in return change the vectorial capacity of mosquitoes [30]. However, when free-flying mosquitoes were exposed to coils under outdoor conditions in the SFT, there was no effect on successive blood feeding as depicted by similar proportions of blood fed mosquitoes in the control and treatment. The different results observed in the Peet Grady chamber may be attributed to limited ventilation that resulted in increased insecticide particles per area and a higher dose of insecticide obtained by the mosquito, hence the enormous reduction in blood feeding. The effect of coils in the SFT was less pronounced probably because of the large surface area of the facility and increased airflow, consequently a low dose of Transfluthrin was distributed within a large area and mosquitoes contacted much lower doses that did not affect their feeding. It should be noted that the coils used in the SFT contained the standard dose of Transfluthrin (0.03%) that is meant for indoor use. Therefore increasing the dose is likely to enhance efficacy of the coils. These results emphasize the need to consider the dose of the active ingredient required to confer maximum protection for different settings such as indoors and outdoors.

7.1.4. Location of repellent and its efficacy

Coils used as a “point source” reduced bites by almost half when coils were placed 0.3m away from the human (Chapter 5). Interestingly the “bubble” reduced bites by more than three quarters when coils were placed 0.3m away from the human (Chapter 5). The “bubble” reduced bites by approximately 40% when coils were placed between 1m - 15m from the human compared to the “point source” where bites were reduced by less than 27% when coils were placed between 1m - 15m. This study highlights the need to consider presentation of the source of the active ingredient around humans. In order to achieve maximum efficacy, there should be a chemical barrier between the human and mosquitoes. The “bubble” provided a chemical barrier in all directions around the human and ensured maximum saturation of the space with the active ingredient.

It should be noted that the *An. gambiae s.s.* mosquitoes used in the semi-field studies were anthropophilic, endophagic and endophilic. Therefore these results should be regarded with caution. This study indicates that SRs are likely to have pronounced effects on human biting rate and indoor mosquito densities where mosquitoes bite and rest indoors. This is due to high irritancy that forces mosquitoes to leave houses prematurely as well as reduction in feeding.

Unfortunately this study could not measure irritancy on exophilic *An. arabiensis* mosquitoes in field experimental huts. This is because mosquitoes were removed from experimental huts at the top of every hour and thus there was no way of knowing how many mosquitoes were left inside the hut at a specific time. This meant that the proportion of mosquitoes that left huts was known but the proportion of the ones that did

not leave was unknown. It is worthwhile noting that mortality reported in the semi-field was a lot higher (66% by Transfluthrin and 61% by Metofluthrin coils and 64% by DDT) than the field (0.02% overall mortality). These discrepancies can be explained by the fact that in field experimental huts human baits slept under untreated bed nets while in the semi-field, mosquitoes were allowed to feed freely on unprotected humans. It is hypothesized that in the semi-field, mosquitoes likely spent more time inside huts because they were tempted to blood feed on unprotected individuals and the time spent inside huts was sufficient to pick up lethal insecticides. In the field, mosquitoes might have left earlier than usual due to irritation of the insecticides and inability to blood feed. A study by Miller and Gibson indicates that despite irritancy caused by Permethrin on bed nets, mosquitoes spent more time on nets due to the need to feed [21]. This might have happened in the semi-field experiments too, leading to high mortality of mosquitoes. It should be noted that laboratory reared *An. gambiae* s.s. mosquitoes were used in the semi-field compared to wild ones in the field, this might have also contributed to a lesser extent to high mortality. However this is highly unlikely because mortality was measured with reference to the control in the particular experiment.

Spatial repellents can be used outdoors for personal protection. In this case they would reduce human biting rate only, and are unlikely to be used at concentrations high enough to induce mortality due to the high cost of the compounds and toxicity accompanied with high doses of insecticide that might render them unsafe for humans or non-target organisms. It is necessary to conduct further studies in different geographical settings in order to determine the effect of SR on mosquitoes of different behaviours and the effect on malaria transmission as well as nuisance mosquitoes because efficacy against these

mosquitoes will encourage uptake of the tool and regular use by the community.

It is important to consider the overall mode of action of SR on the mosquito population structure and the behaviour. The effect of LLINs on mosquito behaviour was not considered until recently when it has been shown that high coverage may have prompted species shift or behaviour change of mosquitoes [31]. Therefore it seems worthwhile to consider the effect that long-term, widespread use of SR would have on mosquito behaviour as early as possible. This study highlights subtle effects of SR such as the effect on feeding behaviour and fecundity. Sufficiently high doses of SR delay the resumption of the normal feeding process of mosquitoes, but mosquitoes can resume feeding after 12 hours – they do not become refractory. This might shift the feeding cycle of mosquitoes forward to different biting times as has been observed with LLINs [32]. By preventing man-vector contact LLINs can disrupt the feeding process of mosquitoes hence increase the length of the oviposition cycle of the overall population. This mechanism might explain the immediate change in biting cycles of both *An. farauti* and *An. koliensis* after LLINs distribution in Papua New Guinea [32]. Another study also showed that females that failed to obtain blood during the previous night were likely to start host seeking early in the evening of the next day [33]. Such studies conducted at large scale under natural settings would be beneficial in the long run if SR will be proven to complement existing tools by reducing malaria prevalence and transmission.

This study highlights the need for standardizing testing procedures for airborne chemicals. Testing conditions such as temperature, humidity, wind speed, wind direction and the presence or absence of the human influence the results. The WHOPES recently published guidelines for testing SR[9]. They include laboratory studies which provide

information on critical outcome measures for testing active ingredients comprising; movement of mosquitoes away from chemicals, host attraction-inhibition as well as dose-response relationships. The guidelines also include a section on testing formulations in semi-field trials. In the semi-field trials experiments can be conducted using free-flying pathogen free mosquitoes in screened enclosures [9]. Some of the experiments reported in this study were conducted according to the semi-field trial guidelines. Feeding inhibition, irritancy and excito-repellency were measured accurately in experimental huts enclosed in a screen house (Chapter 5).

7.2. The effect of spatial repellents on malaria transmission

Mosquito control measures are aimed at interrupting disease transmission by significantly reducing vectorial capacity over a prolonged period of several years to induce disease interruption. Effective vector control tools target several stages of the mosquito's feeding cycle thereby reduce the probability of mosquitoes to transmit diseases. According to the vectorial capacity equation, changes to different aspects of the life cycle of mosquitoes will have differential impact on malaria transmission [34]. A reduction in mosquito density (m) leads to an equal reduction in vectorial capacity because of their linear relationship, while a reduction in biting rate (ma) leads to a two-fold reduction in transmission due to the quadratic relationship (arising from the fact that mosquitoes need to feed twice to transmit malaria: once to become infected and once to infect) [34]. This study shows that through deterrence, irritancy and feeding inhibition of SR, more than 90% of the mosquitoes are prevented from contacting humans inside houses before

mortality occurs. By reducing human-vector contact, SR directly influence the biting rate of mosquitoes (ma): an important parameter of malaria transmission. In addition results from the semi-field system indicate that SR lead to delayed mortality of mosquitoes and therefore affect mosquito densities (m) and indirectly reduce chances that a mosquito would survive (p) long enough to become infectious. This study also shows that airborne pyrethroids reduce fitness of mosquitoes by reducing the number of eggs laid. Reduced fecundity is an indirect measure of pyrethroids on mosquito densities (m). This study suggests that SRs are likely to have greater impact on malaria transmission than initially considered because they influence more than one parameter of the vectorial capacity equation. This study underlines entomological parameters that are affected by SR and highlights the need for further field studies to confirm the results and demonstrate epidemiological impact.

7.3. Where do SR fit in the malaria vector control strategies?

The WHO currently recommends diagnosis of malaria cases and treatment with effective medicines; distribution of LLINs, to achieve full coverage of populations at risk of malaria; and IRS to reduce epidemic transmission and eliminate malaria at low transmission [35]. In endemic areas everyone should sleep under an LLIN irrespective of the age group [36]. LLINs are highly effective where mosquitoes are highly anthropophilic, endophagic, endophilic and bite late at night. Increasing reports of changes in mosquito behaviour or species shift of malaria vectors threaten the efficacy of bed nets. Mosquitoes have been shown to exhibit a range of behaviours that may attenuate

efficacy of LLINs including early evening and outdoor biting, exophily and avoidance of insecticide treated houses [31].

7.3.1. Outdoor mosquito control

Currently there are no vector control tools that target outdoor biting mosquitoes apart from insecticide treated materials such as pyrethroid treated clothing, hammock nets and use of topical repellents that are being used for personal protection against outdoor biting mosquitoes. Among these tools, topical repellents [37, 38] and long lasting insecticidal hammocks [39] have been shown to reduce malaria prevalence. Topical repellents are especially effective for personal protection but the effect on malaria control in a community may be undermined where there is minimal coverage and minimal compliance by users. This is because topical repellents prevent mosquitoes from feeding. Therefore mosquitoes that don't feed are likely to be diverted to non-users of the repellent [40]. Larviciding may also be used to control outdoor biting mosquito densities through larval source management where larval habitats can be identified and targeted. However it is likely to be inappropriate for rural settings in most parts of Africa where there are numerous larval habits that are not easy to identify and may be left out during treatment [41]. This study indicates that SR may be useful against early evening and outdoor biting mosquitoes because unlike personal protection tools they are likely to offer household protection due to the area wide effect which extends over several distances up to 15 meters. In addition to reduction of human biting rate SR used indoors also delay future mosquito feeding episodes and hence, they are likely to prevent diversion to non-users and also reduce mosquito fitness by reducing fecundity. Therefore long-lasting

passive SR are likely to be superior to personal protection tools for community or programmatic control because they affect more than just the human biting rate of mosquitoes [10].

7.3.2. Combination with other control tools

Mathematical modeling suggests that adding SR indoors in the same space as LLINs may reduce their efficacy. It is suggested that SR through excito-repellency may prevent mosquitoes from reaching bed nets and acquiring lethal doses [42]. This underscores the need to determine the effect of SR in the presence of LLINs as well as IRS. Laboratory and semi-field trials should be conducted to evaluate the effect of using SR products alongside LLINs and IRS that are treated with different active ingredients. Different techniques of combining SR with existing tools without influencing their efficacy should be developed. For instance SR may be used exclusively outdoors where mosquitoes are exophagic and exophilic when people are outdoors at the same times when vectors are active there. Another method may be combining a SR and an LLIN. A novel long lasting polymer-coating multi-layer technique was previously used to combine different repellents with pyrethroid treated nets. DEET combined with a permethrin treated bed net increased knock down, contact toxicity, spatial repellency and also reduced biting [43]. This technique is likely to be useful for adapting use of SR for malaria vector control. Lessons may be learnt from studies conducted to determine the effect of combining LLINs and IRS or durable wall linings [44-47]. Combining LLINs and non-pyrethroid

durable wall lining was shown to be more effective than using pyrethroid durable wall linings and the combination of interventions was less likely to select for resistance [47].

7.3.3. Resistance management

Resistance against pyrethroids has been reported in many endemic areas [48]. Increased use of pyrethroids through use of spatial repellent is likely to fuel development of pyrethroid resistance. May be it is worthwhile to develop other SR from new compounds that are not pyrethroids in order to lessen the burden of insecticide resistance. Despite this, a study carried out in Benin indicated that coils were effective against highly kdr resistant *Cx. quinquefasciatus quinquefasciatus* (Raphael N'Guessan pers. comm). Efficacy of SR against resistant mosquitoes may be explained by the fact that airborne repellent pyrethroids modulate the action of numerous odorant receptors and lead to multiple behavioral responses of mosquitoes that include reduced feeding and avoidance [27]. The target sites for repellent modes of action of pyrethroids are different from those involved in toxic effects that result in knock down and mortality of mosquitoes and this is to which insecticide resistance has developed. The most important question is whether resistance is likely to develop towards the anti-feeding and avoidance effects elicited through the effect of repellent pyrethroids on odorant receptors.

7.3.4. Community studies

It is important to consider mosquitoes that are prevented from feeding. Where do they go? Are they diverted to non-users leading to increased infectious bites? A study

conducted in Kilombero valley, Tanzania indicated that topical repellents that also prevented biting, increased the density of mosquitoes to nearby non-users by four fold [40]. Spatial repellents may be of minimal risk because apart from reducing feeding instantly, they delay feeding for hours and also induce mortality of mosquitoes. Nevertheless, it is necessary to conduct further studies that measure whether SR result to diversion when used at a large scale in the community.

It is essential to not only develop SR products that are effective but also those that are readily acceptable by the targeted user. It is important to determine the preferences and choice of SR products. This will enable development of suitable delivery formats that are desirable and therefore increase compliance by users. In developing countries, there is need to develop affordable tools that do not require an external source of energy such as electricity. Paper emanators impregnated with metofluthrin have been shown to be effective against indoor and outdoor biting mosquitoes [49-51]. Development of emanators that last for several weeks or months is underway. In this study a delivery format for dispensing Transfluthrin from hessian strips was developed and evaluated [52]. Transfluthrin treated hessian strips reduced bites by more than 90% for more than 6 months without retreating the fabric [52]. Long-term efficacy of the strips may be attributed to the high dose of Transfluthrin used. Two percent Transfluthrin (10ml of Transfluthrin/500ml of the solvent) was impregnated on a 4 x 0.3m long strip. The strips were used outdoors therefore the risk of users inhaling toxic amounts was lower due to increased airflow. However, toxicology studies giving a No Observed effect Level for chronic outdoor exposure will be needed before this product can be adapted for inclusion in vector control strategies. This information is useful in determining the minimal active

ingredient required to provide protection against mosquito bites as well as ensure safety for users. Application of the strip for use in a household was heavily criticized because the strips were presented in close proximity to users and which increased the risk of skin contact with the treated material. Further studies are being conducted with the aim to modify current strips into more user-friendly formats. The new formats will include reduced doses of Transfluthrin as well as improved formats that prevent direct skin or oral contact with chemical. If proven effective, the new formats will be a great tool for protecting people whilst outdoors in verandas especially in coastal tropical areas where evening and night temperatures are quite high; hence people rest, play, cook or sleep outdoors. The tools can also be used in outdoor restaurants in the early evening and late at night or by night watchmen.

7.4. Conclusion

This study elucidates the mode of action of SR. Spatial repellents mainly interfere with host seeking and thus ultimately prevent mosquitoes from blood feeding. This information is critical for the development of target product profiles for spatial repellent products.

This study highlights the potential of SR for mosquito and malaria control and underlines several important entomological parameters that should be quantified in a proof of concept clinical trial in order to effectively determine the impact of SR on malaria epidemiology.

This study distinguishes between taxis and orthokinesis and reveals that airborne Transfluthrin elicits orthokinesis and do not prevent attraction of mosquito to humans but prevent them from blood feeding. This study also indicated that coils provide area wide protection and provided insights on how to use SR to provide a chemical “bubble that provides maximum efficacy. In addition, this study demonstrates that locally available natural fibres such as hessian/sisal [52] are promising absorbent substrates for dispensing volatile insecticides, such as Transfluthrin, without the need for electricity and they are likely to protect people against outdoor biting mosquitoes in tropical settings. Spatial repellents may be a suitable complementary option where mosquitoes feed in the early evening and/or rest outdoors. The role of SR in integrated approach of malaria control should be critically considered with an aim of complementing existing mainstream tools rather than undermining existing control efforts.

Glossary

Anthropophilic: Tendency of hematophagous arthropods to prefer human hosts [53].

Attraction inhibition: Compounds with an effect that results in a reduction of the number of organisms that respond to an attractive stimulus [54]. In the case of insects this is accomplished by inhibition or excitation of olfactory receptor neurone responses [55].

Behavioristic avoidance: Also known as behaviouristic resistance or protective avoidance- modified behaviour whereby endophilic mosquito populations sometimes adapt to exophily in response to pressure of indoor residual spraying with excitorepellent insecticide [53].

Deet: N,N-diethyl-3-methylbenzamide (originally known as *N,N-diethyl-meta-toluarnide*), usually abbreviated to deet or deet in literature. It is the dominant repellent used worldwide since the 1960s [53].

Deterrence: In the repellent context, something that inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit [5]. It also refers to when mosquitoes are prevented from entering insecticide treated houses [56, 57].

Diversion: Movement of a haematophagous arthropod from a protected to unprotected target caused by the use of repellents [58].

Endophilic: Tendency of insects (especially female *Anopheles* mosquitoes of some species) to come into houses for biting nocturnally and resting diurnally [53].

Exophilic: Behavioral tendency of female insects to stay outdoors [53].

Excito-repellency: A chemical that causes insects to make undirected movements that set them apart from the source [5]. This is due to a combination of orthokinesis undirected changes in the speed and klinokinesis undirected changes in the rate of turning of an organism depending on the intensity of the stimulus. When exposed to excito-repellent insecticides such as DDT mosquitoes tend to move towards the light resulting in escape from treated houses [14].

Feeding inhibition: An inhibitor is a compound that suppresses the action with another compound. Several repellents have been shown to suppress insect attraction to a resource, e.g. inhibitor of attraction [22]. Therefore feeding inhibition is whereby mosquitoes are prevented from biting and/or feeding on humans [56].

Irritancy: The terms irritancy and excito-repellency are used interchangeably to refer to increased undirected activity of mosquitoes through orthokinesis or klinokinesis arising after 1) tarsal contact with insecticide treated surfaces “contact irritancy” or 2) airborne insecticides, “non-contact irritancy” [4].

Knockdown: Sublethal incapacitation; early symptom of an insect responding to a pesticide; not necessarily lethal because metabolic recovery may occur [53].

Olfactory receptor Perception of chemicals in the environment by insects begins when compounds activate ionotropic receptors (IR), gustatory receptors (GR) and odorant receptors (ORs) located on the dendritic surface of olfactory receptor neurons (ORNs), chemosensory neurons housed in a head appendage (e.g. antenna or palp) [59]. Olfactory receptors recognize biologically meaningful chemical ligands, governing their sensitivity and specificity thus regulating innate and learned olfactory behaviours including

attraction and repellency [60]. The expression of ORs follows the general rule of one OR to one ORN. Rather than binding specific ligands, olfactory receptors may display an affinity for a range of odour molecules, and conversely a single odorant molecule may bind to a number of olfactory receptors with varying affinities, with some such as pheromone receptors showing high affinities (specificities). The insect odorant receptors are atypical 7-transmembrane domain proteins that form ligand-gated ion channels by assembling a ligand-selective subunit with the olfactory correceptor Orco [61].

Orthokinesis: Irritation effect which causes insects to move undirected to stimulus gradient and increase their activity with the result that there would be a decreased number on the surface [5].

Pyrethroids: Numerous synthetic organic compounds, mostly based on the chrysanthemate moiety of pyrethrum, having analogous neurotoxic modes of action causing rapid knockdown and insecticidal effects [53].

Repellent: a chemical that causes insects to make oriented movements of avoidance without having made actual tarsal contact with the chemical stimulant [5].

Spatial repellent: refers to all mosquito behavioural reactions induced by any airborne chemicals that cause mosquitoes to eventually sit apart from the source of stimulation [6].

Taxis: Immediate directional avoiding reaction [5].

Vectorial capacity: the expected number of new human malaria infections disseminated per human per day, by a mosquito population from a single case, presuming that all vector females feeding on the case become infective [62].

Zoophilic: Tendency of hematophagous insects to bite or prefer hosts other than humans [53].

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STUDENT ID NO: 257850

CANDIDATE'S SIGNATURE *Barasa* Date 28th March 2015

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Pesticide Fact Sheet

| | |
|-----------------------------|-------------------------------------|
| Name of Chemical: | Metofluthrin |
| Reason for Issuance: | New Chemical Nonfood Use |
| Date Issued: | September 2006 |

Description of Chemical

| | |
|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| IUPAC name: | 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (EZ)- (1RS,3RS;1RS,3SR)-2,2-dimethyl-3-prop-1- enylcyclopropanecarboxylate |
| CAS name: | [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl 2,2-dimethyl-3-(1-propenyl)cyclopropanecarboxylate |
| Common Name: | Metofluthrin |
| Empirical Formula: | C ₁₈ H ₂₀ F ₄ O ₃ |
| EPA Chemical Code: | 109709 |
| Chemical Abstracts Service (CAS) Number: | 240494-70-6 |
| Chemical Class: | Pyrethroid ester |
| Registration Status: | New Chemical, nonfood use |
| Pesticide Type: | Insecticide repellent not applied to human skin |

U.S. Technical Registrant : Sumitomo Chemical Company, LTD.
1330 Dillon Hghts. Ave.
Baltimore, MD 21228

Use Pattern and Formulations

Currently there are two end use products being proposed for metofluthrin.

DeckMate™ Mosquito Repellent Strip is an impregnated paper strip (~3,528 cm²) containing 1.82 percent metofluthrin as the active ingredient. The product also contains Bitrex™ to discourage oral exposure to children or animals. The product is for use on patios, campsites, decks, cabanas, and other outdoor areas. One strip is applied per 10 ft × 10 ft outdoor area. Indoors the application rate is two strips per 50 m³. There are approximately 200 mg of metofluthrin initially in the strip. The strips can provide up to one week of protection. Metofluthrin evaporates readily and therefore requires no external heat.

Norm 1- is a personal outdoor insect repellent product consisting of a holder containing a replaceable cartridge insert coated with up to 50 mg of metofluthrin. The product is activated by turning on a battery powered fan to release the metofluthrin into the air surrounding the individual. The device can be worn by adults or children for up to 12 hours although a specific time is not presented on the proposed label. A time of 12 hours was used in the exposure study and was used by the Agency. There are no label restrictions on who can use the products or the use frequency.

There are no proposed agricultural or occupational uses for metofluthrin.

Science Findings

Available product chemistry data supporting the use of flufenoxuron are summarized below in Tables 1 and 2.

| TABLE 1 Nomenclature and Physiochemical Properties of Metofluthrin | |
|--------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Chemical Structure | |
| Empirical Formula | C ₁₈ H ₂₀ F ₄ O ₃ |
| Common name | Metofluthrin |
| Company experimental name | S-1264 |
| IUPAC name | 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (<i>EZ</i>)-(1 <i>RS</i> ,3 <i>RS</i> ;1 <i>RS</i> ,3 <i>SR</i>)-2,2-dimethyl-3-prop-1-enylcyclopropanecarboxylate |
| CAS name | [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl 2,2-dimethyl-3-(1-propenyl)cyclopropanecarboxylate |
| CAS Registry Number | 240494-70-6 |
| End-use product/EP | SumiOne®, Eminence® |
| Chemical Class | Pyrethroid ester |

| TABLE 2 Physiochemical Properties of Technical Grade Metofluthrin | |
|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular Weight | 360.34 |
| Melting point/range | NA |
| pH | 5.24 at 25°C (1% aqueous solution) |
| Density | 1.21 at 20°C |
| Water solubility (20°C) | 0.67 mg/L (20°C) for (S-1264RTE) 0.50 mg/L (20°C) for (S-1264RTZ) |
| Solvent solubility (20°C to 25°C) (g/L) | Acetone 303.4, methanol 312.2, ethyl acetate 307.6, toluene 326.9, n-hexanes 328.7, dichloromethane 318.9, n-octanol 325.1, isopropyl alcohol 313.2 |
| Vapor pressure (25°C) | 1.47x10 ⁻⁵ Torr |
| Dissociation constant, pKa | Could not be measured |
| Octanol/water partition coefficient, logP _{OW} (25°C) | 5.03 (S-RTE) 4.97 (S-RTZ) |
| UV/visible absorption spectrum | In 100% methanol: peak maximum = 273 nm, extinction coefficient = 1670 M ⁻¹ cm ⁻¹ , band width 23 nm |

TOXICOLOGY SUMMARY

The Registrant submitted the studies listed in Tables 3 and 4, which include a number of

toxicity studies. These include the usual acute studies for metofluthrin technical. The Registrant has also submitted oral, dermal and inhalation studies as well as chronic, carcinogenicity and developmental studies as shown in Table 4.

| TABLE 3 Acute Toxicity Profile – Test Substance | | | |
|--------------------------------------------------------|-----------------|-------------------------------------------|--------------------------|
| Guideline No./ Study Type | MRID No. | Results | Toxicity Category |
| 870.1100 Acute oral toxicity | 46406719 | LD ₅₀ > 2000 mg/kg | III |
| 870.1200 Acute dermal toxicity | 46406721 | LD ₅₀ >= 2000 mg/kg | III |
| 870.1300 Acute inhalation toxicity | 46406723 | LC ₅₀ > 1.08 and < 1.96 mg/L | III |
| 870.2400 Acute eye irritation | 46406724 | Not an eye irritant | IV |
| 870.2500 Acute dermal irritation | 46406724 | Mildly irritating to the skin (PDI = 0.8) | IV |
| 870.2600 Skin sensitization | 46406726 | Not a dermal sensitizer | - |

| Table 4 Subchronic, Chronic, and Other Toxicity Profile | | |
|------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Guideline No./ Study Type | MRID No. (year)/ Classification /Doses | Results |
| 870.3100 90-Day oral toxicity rats (Wistar rats) | 46454109 (2003) Acceptable/Guideline 0, 100, 300, 1000, or 2500 ppm M: 0, 6.8, 20.6, 70.4, or 183.6 mg/kg/day F: 0, 7.5, 21.6, 73.0, or 185.6 mg/kg/day | NOAEL = 20.6/21.6 mg/kg/day LOAEL = 70.4/73.0 mg/kg/day, based on increased absolute and relative liver weights in both sexes; increased serum total cholesterol and phospholipids levels in males, and increased incidences of enlarged livers, hepatocellular hypertrophy, and basophilia in males; and decreased body weight gain in females. |
| 870.3100 Subchronic (6-month) oral toxicity rats (Sprague-Dawley rats) | 46406733 (2002) Acceptable/Guideline 0, 100, 300, 1000, or 3000 ppm M: 0, 5.3, 16.0, 54.1, 164.6 mg/kg/day F: 0, 6.4, 19.0, 65.4, 191.4 mg/kg/day | NOAEL = 16.0/19.0 mg/kg/day LOAEL = 54.1/65.4 mg/kg/day, based on increased relative liver weights, serum phospholipids, and total cholesterol levels in males; increased incidences of dark, enlarged livers and hepatocellular hypertrophy in both sexes; and an increased incidence of slight focal hepatic necrosis in females. |

| Table 4 Subchronic, Chronic, and Other Toxicity Profile | | |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Guideline No./ Study Type | MRID No. (year)/ Classification /Doses | Results |
| 870.3100 90-Day oral toxicity in mice (CD-1 mice) | 46454108 (2004) Acceptable/Guideline 0, 100, 1500, 2500, or 3500 ppm M: 0, 13.7, 20.9, 35.7, or 48.7 mg/kg/day F: 0, 17.2, 25.2, 43.9, or 58.7 mg/kg/day | NOAEL = 35.7/43.9 mg/kg/day LOAEL = 48.7/58.7 mg/kg/day, based on findings indicative of hepatotoxicity including increased absolute and relative liver weights in both sexes; increased serum total cholesterol, phospholipids, and triglycerides in females; and minimal degeneration/necrosis of the liver and minimal to moderate hepatocellular hypertrophy in both sexes, and increased Kupffer cells in males. |
| 870.3150 90-Day oral toxicity in dogs (Beagles) | 46406734 (2002) Acceptable/Guideline 0, 10, 30, or 100 mg/kg/day | NOAEL = 30 mg/kg/day LOAEL = 100 mg/kg/day, based on tremor and vomiting observed in both sexes |
| 870.3250 90-Day dermal toxicity in rats (Sprague-Dawley) | 46556101 (2004) Acceptable/Guideline 0, 30, 100, 300, or 1000 mg/kg/day | Systemic NOAEL = 300 mg/kg/day Systemic LOAEL = 1000 mg/kg/day, based on mortality and clinical signs (tremor and salivation) Dermal NOAEL = not determined Dermal LOAEL = 30 mg/kg/day, based on hyperactivity and vocalization in the females during the daily exposure period |
| 870.3465 Subchronic inhalation study in rats (Sprague-Dawley) | 46406736 (2002) Acceptable/Guideline 0, 10, 50, 100, or 200 mg/m ³ 0, 0.01, 0.051, 0.099, or 0.196 mg/L) M: 4 hrs/day, 28 days F: 4 hrs/day, 29 days | NOAEL = 0.099 mg/L LOAEL = 0.196 mg/L, based on mortality and clinical signs including tremors, hypersensitivity, ataxic gait, tiptoe gait, lateral position, clonic convulsion, and hypothermia in both sexes. Clinical signs began on days 1-4 and occurred consistently in the males and transiently in females thereafter. |
| 870.3700a Prenatal developmental in rats (Sprague-Dawley) | 46454111 (2002) Acceptable/Guideline 0, 5, 15, or 30 mg/kg/day from GD6 – GD19 | Maternal NOAEL = 15 mg/kg/day Maternal LOAEL = 30 based on increased incidence of tremor Developmental NOAEL = 30 mg/kg/day Developmental LOAEL = not observed |
| Prenatal developmental in rats (Sprague-Dawley) | 46454112 (2002) Acceptable/Non-Guideline M: 0, 10, or 20 mg/kg/day beginning 2 weeks prior to mating through necropsy (57 days) F: 0, 10, 20, or 40 mg/kg/day beginning 2 weeks prior to mating through GD7 | Parental NOAEL = 20 mg/kg/day (both sexes) Parental LOAEL = 40 mg/kg/day, based on mortality and incidences of tremors and salivation in females. Reproduction NOAEL = 20/40 mg/kg/day M/F Developmental NOAEL = 40 mg/kg/day in females Developmental LOAEL = not observed |
| Prenatal developmental in rats (Sprague-Dawley) | 46454113 (2002) Acceptable/Non-guideline 0, 5, 15, or 30 mg/kg/day from GD6 through LD20 | Maternal NOAEL = 15 mg/kg/day Maternal LOAEL = 30 mg/kg/day, based on mortality and increased incidences of tremors and salivation. Reproductive NOAEL = 30 mg/kg/day Reproductive LOAEL = not observed Developmental NOAEL = 30 mg/kg/day Developmental LOAEL = not observed |

| Table 4 Subchronic, Chronic, and Other Toxicity Profile | | |
|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Guideline No./ Study Type | MRID No. (year)/ Classification /Doses | Results |
| 870.3700b Prenatal developmental in rabbits (New Zealand White) | 46454114 (2002) Acceptable/Guideline 0, 25, 125, or 250 mg/kg/day from GD6 – GD27 | Maternal NOAEL = 25 mg/kg/day Maternal LOAEL = 125 mg/kg/day, based on mortality Developmental NOAEL = 250 mg/kg/day Developmental LOAEL = not observed |
| 870.4100a Chronic toxicity rodents (Wistar rats) | 46611301 (2005) Acceptable/Guideline 0, 20, 200, 900, or 1800 ppm M: 0, 0.8, 8.2, 38.1, or 77.8 mg/kg/day F: 0, 1.0, 10.1, 47.4, or 96.1 mg/kg/day | NOAEL = 8.2/10.1 mg/kg/day LOAEL – 38.1/47.4 mg/kg/day, based on decreased body weights and body weight gains in both sexes; increased incidence of hepatic clear cell foci in both sexes; increased fatty liver change, and kidney lesions (including interstitial fibrosis, lipofuscin, mononuclear foci, and glomerulosclerosis) in males; increased centrilobular hepatocellular hypertrophy in females |
| 870.4100b Chronic toxicity dogs (Beagle) | 46454110 (2004) Acceptable/Guideline 0, 10, 30, or 100 mg/kg/day | NOAEL = 10 mg/kg/day LOAEL = 30 mg/kg/day, based on increased incidence of tremor in males. |
| 870.4300 Carcinogenicity mice (CD-1 mice) | 46611302 (2005) Acceptable/Guideline 0, 100, 1000, or 1750/2500 ppm M: 0, 12, 116, or 209 mg/kg/day F: 0, 15, 155, or 277 mg/kg/day | NOAEL = 116/155 mg/kg/day LOAEL = 209/277 mg/kg/day, based on decreased body weight gain in both sexes. |
| Gene Mutation 870.5100 Bacterial Reverse Gene Mutation Assay | 46406742 (2002) Acceptable/Guideline 0, 156, 313, 625, 1250, 2500, or 5000 µg/plate +/- S9 in <i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537 and <i>E. Coli</i> WP2 uvrA | There was no evidence of induced mutant colonies over background levels. |
| Gene Mutation 870.5100 <i>In vitro</i> Bacterial Gene Mutation Assay | 46454115 (2004) Acceptable/Guideline Trial 1 (-S9): 4.88, 9.77, 19.5, 39.1, 78.1, or 156 µg/plate strains TA100, TA1535 Trial 2 (+S9): 19.5, 39.1, 78.1, 156, 313, or 625 µg/plate strains TA100, TA1535, and TA1537 Trial 3 (+/-S9): 156, 313, 625, 1250, 2500, or 5000 µg/plate strains TA98 and WP2uvrA | There was no evidence of induced mutant colonies over background levels. |

| Table 4 Subchronic, Chronic, and Other Toxicity Profile | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Guideline No./ Study Type | MRID No. (year)/ Classification /Doses | Results |
| Cytogenetics 870.5375 <i>In vitro</i> Mammalian Cytogenetics (Chromosomal Aberration Assay in Chinese Hamster Lung Fibroblasts) | 46406744 (2002) Acceptable/Guideline Trial 1 (-S9): 50, 70, 90, 110, or 130 µg/mL Trial 1 (+S9): 50, 100, 150, 200, or 250 µg/mL Trial 2 (-S9) 20, 50, 80, or 110 µg/mL Trial 2 (+S9): 100, 150, 200, or 250 µg/mL | There was no evidence of chromosome aberration induced over background in the presence or absence of S9-activation. |
| Other Effects 870.5395 <i>In vivo</i> Mammalian Cytogenetics – Erythrocyte Micronucleus Assay in Mice | 46406745 (2002) Acceptable/Guideline 0, 12.5, 25, or 50 mg/kg | There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls. |
| 870.6200a Acute neurotoxicity screening battery (Sprague-Dawley) | 46406728 (2004) Acceptable/Guideline 0, 20, 50, or 100 mg/kg | NOAEL = 50 mg/kg LOAEL = 100 mg/kg, based on mortality, adverse clinical signs, FOB (unusual behavior, limb twitches/tremors, and abnormal respiration) effects, and increased motor activity in both sexes. |
| 870.6200b Subchronic neurotoxicity screening battery (Sprague-Dawley) | 46406729 (2004) Acceptable/Guideline 0, 300, 1000, or 3000 ppm M: 0, 18.3, 59.8, or 178.8 mg/kg/day F: 0, 20.9, 68.8, 206.0 mg/kg/day | Systemic NOAEL = 59.8/68.8 mg/kg/day Systemic LOAEL = 178.8/206.0 mg/kg/day, based on mortality (females only); clinical signs (soft/liquid feces and scant feces in the males and tremors and twitches in the females); decreased body weight, body weight gain, and absolute and relative food consumption in both sexes. Neurotoxicity NOAEL = 59.8/68.8 mg/kg/day Neurotoxicity LOAEL = 178.8/206.0 mg/kg/day, based on the clinical signs of tremors and twitches in the females |
| 870.7485 Metabolism and pharmacokinetics | 46406746, 46406747, 46406748, 46414002, and 46414003 (2004) Acceptable/Guideline 1 or 20 mg/kg for single dose studies 1 mg/kg for 21 day studies | Overall recoveries were 95-97% for both dose groups. Absorption was rapid (detectable plasma residues within 30 minutes, T _{max} 3.3-8.0 hours) and thorough (>80% absorption). Absorption was not dose limited. At 168 hours post dosing, urinary and fecal excretion accounted for 29-71% and 25-66% of the total administered dose, respectively. Radioactivity increased above plasma levels in both liver and kidney, but dissipated 12 hours post-dose. 46 metabolites were identified, including all major metabolites. |
| Non-Guideline An evaluation of the human relevance of the metofluthrin- induced liver tumors in rats based on mode of action | 46756304 (2006) Acceptable/Nonguideline | Summary of proposed MOA and weight of the evidence. The MOA for metofluthrin-induced liver tumors is postulated to involve liver cytochrome P450 enzyme induction leading to stimulation of increased cellular proliferation. MOA not accepted by CARC due to insufficient data. |

| Table 4 Subchronic, Chronic, and Other Toxicity Profile | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Guideline No./ Study Type | MRID No. (year)/ Classification /Doses | Results |
| Non-Guideline Study for the mode of action of S-1264 for liver tumor promotion in rats (Wistar rats) | 46581501 (2005) Acceptable/Nonguideline 0, 900, 1800, or 3600 ppm in the diet for 7 days Concurrent recovery group fed basal diet for 7 days following treatment period | Liver morphology and enzyme induction were affected in at 900 ppm and above, as evidenced by increased liver weights, hepatocellular hypertrophy, replicative DNA synthesis in the hepatocytes, induction of CYP 2B and 3A mRNA, and increased expression of CYP 2B. All of these findings were reversible on cessation of treatment. |
| Non-Guideline The 2 nd study of mode of action of S-1264 for liver tumor promotion in rats | 46756301 (2006) Acceptable/Nonguideline 0, 200, 900, 1800, or 3600 ppm in diet for 7 days | Metofluthrin inhibited gap junction interactions (as evidenced by decreased dye transfer) and induced oxidative stress (measured by lipid oxidation and GSH levels). |
| Non-guideline Study for mode of action of S-1264 for liver tumor promotion in rats (in vitro effects of S-1264 on cytochrome P450 activity and mRNA levels) | 46756302 (2006) Acceptable/Nonguideline Rat, mouse, and human hepatocytes were exposed 50 µM metofluthrin for 3 days, and comparative metabolic profiles were examined. | Metofluthrin induced CYP 2B mRNA and 7-pentoxyresorufin O-depentyrase activity in rat and human hepatocytes, but not in mouse hepatocytes, but the induction level was less than that of phenobarbital induction in human hepatocytes. |
| Non-Guideline Gene expression profiling analysis of early phase treatment in the liver from S-1264 treated rats | 46756303 (2006) Acceptable/Nonguideline Wistar rats were exposed to 1800 ppm metofluthrin for 1 week. DNA microarray was used to evaluate gene expression. | The majority of genes upregulated by metofluthrin were GSTs, CYPs, and UDPGTs. In general, this resembled the upregulation of Phenobarbital, only to a lesser degree. |

HAZARD CHARACTERIZATION/ASSESSMENT

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, “Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations.” <http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf>

Human Testing: This risk assessment does not rely on any data from studies in which human subjects were intentionally exposed to a pesticide or other chemical.

Hazard and Dose-Response Characterization

The toxicology database for metofluthrin is complete for the proposed use pattern. Although metofluthrin is a neurotoxicant, a developmental neurotoxicity (DNT) study is not necessary at this time. However, if new uses are proposed, the need for a DNT study

will need to be re-evaluated. The risk assessment team is confident that risk to pregnant women and children will not be underestimated due to: 1) regulatory endpoints are based on neurotoxicity, 2) no neuropathy or changes in morphometrics were observed in the acute and subchronic neurotoxicity studies, and 3) for pyrethroids where DNT studies are available for endpoint consideration, the regulatory endpoints are generally based on neurotoxicity to dogs because dogs are more sensitive to pyrethroids than rats (it is unlikely that a DNT in rats would produce a lower neurotoxicity NOAEL than the NOAEL from the chronic dog study).

Summary and Discussion of Dose Related Effects

Metofluthrin, like other pyrethroids, is neurotoxic in rats, rabbits, and dogs; both sexes were equally sensitive to metofluthrin. Clinical signs include tremor (all species), vomiting (dog only), and increased salivation (rats and dogs). Clinical signs appeared within 206 hours post-dosing and generally disappeared by the dosing period the following day. All routes of exposure (oral, dermal, and inhalation) elicited neurotoxic effects in rats. Rats appeared to be most sensitive via the inhalation route, based on clinical signs including ataxic gait, tremors, tip-toe gait, lateral position, clonic convulsion, hypothermia, and mortality in both sexes. In the acute neurotoxicity battery, neurotoxic effects were seen in rats following a single dose of 100 mg/kg/day including mortality, tremors/twitches, abnormal respiration, and increased motor activity (acute NOAEL = 50 mg/kg/day). Dermal exposure to 10 mg/kg/day in rats produced increased vocalization during the daily application period, which subsided after the removal of the chemical. There were no systemic effects resulting from dermal exposure. In subchronic exposures in rats (based on the subchronic and developmental studies, NOAELs ranged 15-20 mg/kg/day) the LOAELs range from 30-54.1 mg/kg/day, based on liver effects and neurotoxicity. Neurotoxicity was not noted in the chronic studies. The dose-response curve for neurotoxicity is steep with mortality occurring frequently at the LOAEL; death was preceded by tremor, convulsion, salivation, and prostration.

Metofluthrin also targeted the liver in rats and mice, producing increased absolute and relative liver weights, hepatocellular hypertrophy, and increase incidence of enlarged, discolored livers. Hepatocellular toxicity was present at or above 48.7 mg/kg/day in mice and 54.1 mg/kg/day in rats in the subchronic studies. In the chronic rat study, exposure to metofluthrin was connected to increased incidence of hepatocellular adenomas, carcinomas, and combined tumor types at doses greater than or equal to 38.1 mg/kg/day. The registrant submitted a proposed mitogenic mode of action (MOA) for hepatocellular tumor induction. While these studies did suggest a mitogenic MOA was plausible, the studies did not provide enough information for the Agency to accept their proposed MOA. Metofluthrin is not mutagenic or cytotoxic; it does not induce peroxisomal proliferation. The Agency classified this chemical as “likely to be carcinogenic to humans” and generated a Q1* of 1.62×10^{-2} , based on the increased liver tumors in female rats.

In utero and/or post-natal exposure to metofluthrin did not produce any evidence of increased qualitative or quantitative susceptibility in fetuses or pups. Four acceptable developmental studies in rats and rabbits were submitted for metofluthrin. Maternal toxicity was seen at or above 30 mg/kg/day in rats (tremor, salivation, and mortality) and 125 mg/kg/day in rabbits (mortality, preceded by tremor/convulsion). These doses did

not produce any developmental effects on the fetuses or pups. A developmental toxicity study is not being requested at this time for the following reasons: 1) neurotoxicity is well defined within the toxicology database, 2) regulatory endpoints are based on the neurotoxicity, and 3) there were no pathology findings or changes in morphometrics noted in either the acute or subchronic neurotoxicity studies. The FQPA safety factor has been reduced to 1x.

Considerations for Infants and Children

The toxicity database for this chemical is complete for the purposes of this risk assessment. Acceptable neurotoxicity and developmental studies have been submitted for review. Though not required for a non-food use registration, a 2-generation rat reproduction study is being conducted. The Agency has received preliminary results in a 6(a)(2) document, but the final study report has not been submitted at this time.

Neurotoxicity

Evidence of neurotoxicity exists throughout the entire toxicology database via the oral route of exposure in three species (rats, rabbits, and dogs) and via dermal and inhalation routes of exposure in rats.

In the acute neurotoxicity study in rats, a single dose of 100 mg/kg produced tremors, twitches, abnormal respiration, increased motor activity, and mortality. The animals found dead or in extremis 24 hours post-dosing (7 out of 20 animals) exhibited signs of clonic convulsions, hyperpnea, prostration, lost righting reflex, soft or liquid feces, tonic extensor convulsions, salivation, chromorhinorrhea, and chromodachyorrhea. In the subchronic neurotoxicity study, the LOAEL (59.8/68.8 M/F, respectively) was based on mortality in the females; clinical signs including tremors and twitches (in females); decreased body weight and body weight gain, and absolute and relative food consumption in both sexes. Neither study indicated neuropathy.

In a subchronic oral study in rats, all animals exhibited signs of tremor 2-6 hours post-dosing during Week 1 of treatment at doses above 164.6 mg/kg/day; 0-2 animals/sex exhibited transient tremors throughout Weeks 2-3. No clinical signs were observed after Week 3. At 100 mg/kg/day in the subchronic dog study, 5/6 males showed signs of tremor (1-7 incidences/animal) beginning on Day 23 and 5/6 females showed signs of tremor (1-5 incidences/animal) beginning on Day 10. Mild repetitive jerks or tremors of the head, limb or body were seen in 1 animal/sex at Weeks 12-13 (male) and Weeks 11 and 13 (female); these effects were evidenced during cage-side and table top observations. Three developmental rat studies were performed for metofluthrin; all three maternal LOAELs were based on tremor and salivation and two maternal LOAELs included mortality.

In the subchronic dermal study in rats, two females were found dead on Day 2 in the 1000 mg/kg/day dose group. One female, before being found dead, displayed tremors prior to dosing and salivation 3-5 hours post-dosing. Hyperactivity and vocalization were transiently observed in the ≥ 30 mg/kg/day females and ≥ 100 mg/kg/day males during the daily application period on Days 1-4. There were no treatment-related clinical signs outside of the daily dosing period.

In the 28-day inhalation toxicity study in rats, 7/10 males and 3/10 females in the 0.196 mg/L dose group died. At this concentration, tremors of the tail and body were observed during the treatment period; tremor, hypersensitivity, ataxic gait, tiptoe gait, lateral position, clonic convulsion, and hypothermia were observed. Onset occurred on Days 1-4, and clinical signs were transiently seen until Day 26 in males and less frequently in the females until Day 24.

No evidence of neurotoxicity was recorded in either the rat or the mouse chronic/carcinogenicity studies. Increased incidence of tremor was observed in males at 30 mg/kg/day in the chronic dog study. Tremor was observed in the head, limbs, or body of all males beginning on Day 96 (1-5 incidences/dog except one male with 46 incidences) and in only one female and only on Day 289. Tremors were observed 2-6 hours post-dosing and disappeared by the time of observation the next morning.

Developmental Toxicity

Acceptable guideline developmental toxicity studies in rats and rabbits have been submitted for review, along with two acceptable non-guideline developmental studies in rats. In the three rat studies (MRID 46454111, 46454112, 46454113) maternal toxicity was observed in the form of neurotoxicity (tremors and salivation) and death. Neurotoxic effects were observed 2-3 hours post dosing and disappeared by the following day. The maternal NOAELs ranged from 15-20 mg/kg/day, and the maternal LOAELs ranged from 30-40 mg/kg/day. No developmental effects were seen in the rat studies up to 40 mg/kg/day. In one non-guideline study (MRID 46454112) males and females were dosed during the premating and mating periods all the way through gestation day (GD)7 for females. No reproductive effects were noted in either the males or females up to 20/40 mg/kg/day (males/females, respectively, highest dose tested). In the other non-guideline study (MRID 46454113), the female rats were dosed from GD6 (implantation) through lactation day (LD)20. Reproductive effects were not observed in the P or F1 generations. There were no offspring effects noted with regard to FOB results, sensory reflexes, clinical signs, developmental landmarks, body weights, or gross pathology up to the highest dose tested of 30 mg/kg/day.

In the rabbit developmental study, one female in the 125 mg/kg/day group exhibited sneezing and convulsions before death on GD23. One female in the 250 mg/kg/day dose group was found dead on GD14. These deaths were considered treatment related because another female was found dead with convulsions preceding death in the range finding study at 200 mg/kg/day. There were no other mortalities or clinical signs; the LOAEL was determined to be 125 mg/kg/day. The maternal NOAEL is 25 mg/kg/day. There were no treatment-related effects on developing fetuses; the developmental LOAEL was not observed. The developmental NOAEL was determined to be 250 mg/kg/day, the highest dose tested.

Reproductive Toxicity

A reproductive study in rats has not been submitted to the EPA at this time. However, the Agency has received a 6(a)(2) document indicating that a 2-generation reproduction study in rats is being performed. Preliminary findings include neurotoxic effects (tremors, convulsions, and salivation) in the F1 and F2 generations. When the final study report is submitted, a full review of the data will be conducted.

Pre-and/or Postnatal Toxicity

There were no effects on fetal growth or development up to 40 mg/kg/day in rats or 250 mg/kg/day in rabbits; doses at which maternal toxicity was present. There were no treatment related effects on the numbers of litters, fetuses (live or dead), resorptions, sex ratio, or post-implantation loss. There were no effects on fetal body weights or skeletal ossification; and no external, visceral, or skeletal malformations or variations were observed.

Developmental Neurotoxicity

A DNT study is not being requested at this time; however, because this chemical is part of the pyrethroid class, the need for a DNT study will be re-evaluated for all future proposed uses

Summary of Toxicological Doses and Endpoints for Metofluthrin for Use in Human Risk Assessments

| Table 3.4.2 Summary of Toxicological Doses and Endpoints for Metofluthrin for Use in Non-Occupational Human Health Risk Assessments | | | | |
|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Exposure/Scenario | Point of Departure | Uncertainty Factors | Level of Concern for Risk Assessment | Study and Toxicological Effects |
| Incidental Oral Short-Term (1-30 days) | NOAEL = 15 mg/kg/day | UF _A = 10x UF _H = 10x | Residential LOC for MOE = 100 | Developmental Rat Study LOAEL = 30 mg/kg/day based on increased incidence of tremor in maternal animals |
| Dermal Short-Term (1-30 days) | NOAEL = 300 mg/kg/day | UF _A = 10x UF _H = 10x | Residential LOC for MOE = 100 | 90-Day Dermal Rat Study LOAEL = 1000 mg/kg/day based on mortality and clinical signs |
| Inhalation Short-Term (ALL DURATIONS) | NOAEL = 16 mg/kg/day | UF _A = 10x UF _H = 10x | Residential LOC for MOE = 100 | 28-Day Inhalation Study in Rats LOAEL = 32 mg/kg/day based on mortality and clinical signs including tremors, ataxia, hypersensitivity, ataxic gait, tiptoe gait, lateral position, clonic convulsion, and hypothermia in both sexes |
| Cancer (oral, dermal, inhalation) | Likely to be a human carcinogen | Q ₁ * = 1.62×10^{-2} (mg/kg/day) ⁻¹ Dermal absorption factor = 17% | | Based on female rat liver combined adenoma and carcinoma tumor rates |

NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_{DB} = to account for the absence of key data (i.e., lack of a critical study). FQPA SF = FQPA Safety Factor. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

Public Health and Pesticide Epidemiology Data

Metofluthrin is a new active ingredient; therefore, no epidemiological data is available at this time.

Dietary Exposure/Risk Characterization

There are no proposed agricultural uses for metofluthrin at this time; therefore dietary exposure is not expected.

Residential (Non-Occupational) Exposure/Risk Pathway

The aggregate exposure assessment is based solely on residential use patterns. Due to the seasonal nature of insect repellents, only short-term exposure scenarios were considered. The incidental oral endpoint for children was based on maternal neurotoxicity in the rat developmental study (NOAEL = 15 mg/kg/day). This endpoint was selected because of the appropriate time period in which the maternal neurotoxic effects were seen. The short-term dermal endpoint for adults and children (15 mg/kg/day) was selected from the same developmental rat study based on neurotoxic effects because no systemic toxicity was present in the 90-day dermal study. A dermal penetration study in rats was submitted for metofluthrin, which suggests a 17% dermal absorption rate. This 17% dermal absorption rate was applied to all dermal exposure scenarios. The 28-day inhalation study in rats provided a sensitive inhalation endpoint (0.099 mg/L) based on mortality and neurotoxic effects (including tremors, hypersensitivity, ataxic gait, tip-toe gait, clonic convulsion, and hypothermia). The default absorption value of 100% was applied to the inhalation exposure assessment. All levels of concern are set at 100, based on a 10x interspecies extrapolation safety factor and 10x intraspecies variability safety factor. The FQPA safety factor was reduced to 1x.

As a part of every pesticide risk assessment, the Agency considers a large variety of consumer subgroups according to well-established procedures. The Agency estimates risks to population subgroups from pesticide exposure that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, the Agency is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, nondietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas post-application are evaluated. Further considerations are currently in development as the Agency has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific populations.

Estimated Cancer Risk

The Q1* for metofluthrin was based on female hepatocellular adenomas, carcinomas, and

combined adenomas/carcinomas in rats. The Q1* is 1.62×10^{-2} (mg/kg/day)⁻¹. This cancer assessment is conservative in assuming that the product will be used 12 times per year for 50 years out of a 70 year lifespan.

A high-end worst case inhalation cancer assessment was performed for the metofluthrin products (DeckMate and NORM-1). The saturation concentration of 0.28 mg/ m³ was used, with a 12 hour / day exposure time (half a day). An adult breathes 20 m³ of air per day. The use frequency was 12 applications per year from the use survey conducted by the Residential Exposure Joint Venture (REJV). The users are expected to use the products over a 50 year period in their 70 year lifetime. This results in a Lifetime Average Daily Dose (LADD) of 0.000939 mg/kg/day. The LADD is multiplied by the Q1*, which results in an estimated cancer risk of 1.5×10^{-5} .

Aggregate Exposure Assessment

Metofluthrin is proposed for residential use only at this time. No food uses exist. Residues in water are unlikely. An aggregate exposure assessment is not needed at this time.

Cumulative Risk Characterization/Assessment

Metofluthrin is a member of the pyrethroid class of pesticides. Although all pyrethroids alter nerve function by modifying the normal biochemistry and physiology of nerve membrane sodium channels, EPA is not currently following a cumulative risk approach based on a common mechanism of toxicity for the pyrethroids. Although all pyrethroids interact with sodium channels, there are multiple types of sodium channels and it is currently unknown whether the pyrethroids have similar effects on all channels. Nor do we have a clear understanding of effects on key downstream neuronal function e.g., nerve excitability, nor do we understand how these key events interact to produce their compound specific patterns of neurotoxicity. There is ongoing research by the EPA's Office of Research and Development and pyrethroid registrants to evaluate the differential biochemical and physiological actions of pyrethroids in mammals. This research is expected to be completed by 2007. When available, the Agency will consider this research and make a determination of common mechanism as a basis for assessing cumulative risk. For information regarding EPA's procedures for cumulating effects from substances found to have a common mechanism on EPA's website at <http://www.epa.gov/pesticides/cumulative/>.

Occupational Exposure/Risk Pathway

Only residential uses are proposed for metofluthrin at this time; dietary and occupational risk assessments are not necessary at this time.

ENVIRONMENTAL FATE AND EFFECTS SUMMARY

Metofluthrin, like other synthetic pyrethroids, is practically non-toxic to mammals and birds, but it is highly to very highly toxic to aquatic animals and insects. Its repellency power is related to its insecticidal character. The published literature supports its character both as a repellent and as an insecticide. No Level of Concern was exceeded, but the insecticidal properties of metofluthrin imply that it will pose a risk to non-target

insects and to species federally listed as endangered or threatened by the United States government.

Since there is no geographic restriction on metofluthrin's use, it will be used in every place where there are mosquitoes. The proposed use is not expected to stress aquatic or terrestrial vertebrates or aquatic invertebrates even though it is toxic to them, because it is not expected to have a high aquatic concentration.

Environmental Effects

The registrant has submitted adequate effects and fate data needed to complete a Tier 1 Risk Assessment. A summary of all submitted studies are shown in Table 5 and 6 below. Metofluthrin's effect on aquatic organisms is estimated from acute, subacute and chronic laboratory studies submitted to the Agency. The registrant has submitted acute and chronic studies on aquatic vertebrates and invertebrates. Freshwater fish, e.g., bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) are used as surrogates for all freshwater fish species. Freshwater fish are used as surrogates for aquatic-phase amphibians. No acute bluegill sunfish (§72-1a) was submitted. A common carp study was ruled "supplemental," because it is not a standard species. The Agency shall require confirmatory data to satisfy the acute bluegill sunfish data requirement.

The effect of metofluthrin on all bird species is estimated from acute, subacute and chronic studies on two species, bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*). These species also act as surrogates for reptiles and terrestrial-phase amphibians. Effects on mammals are estimated from acute and chronic rat studies submitted to and reviewed by the Agency.

No studies have been submitted that address toxicity to insects. The registrants have requested a waiver for a study on beneficial insects (bees), but this has not been granted. There are no published field surveys or monitoring data. Published information (Kawada, et al.) found that metofluthrin kills insects (mosquitoes) in a cage. All experimental mosquitoes directly under a paper strip were killed within 24-hours. This was not quantified nor was a measure of toxicity (LD50, etc.) calculated. The Agency shall require confirmatory data to satisfy this data requirement.

| TABLE 5 Measures of Environmental Effects of metofluthrin | | |
|-----------------------------------------------------------|--------------------------------|----------------------------------|
| Guidelines | Data Requirements | Measures Of Effect |
| 71-1(a) | Acute Avian Oral Quail or Duck | LD ₅₀ >2250 mg/kg-bw. |
| 71-2(a) | Avian Dietary/Quail | LC ₅₀ >5760 mg/kg-bw |
| 71-2(b) | Avian Dietary/Duck | LC ₅₀ >5760 mg/kg-bw. |
| OPPTS 870.1100 | Rat Acute Oral LD50 | LD50 >2,000 mg/kg |

| | | |
|----------------------|------------------------------------|---------------------------------------------------------------------------------------|
| Non-guideline | Rat reproductive development study | NOAEL = 20 mg/kg bw/day dose based on maternal mortality during the 57 days of dosing |
| 72-1 | Fish Toxicity- Common carp | LC ₅₀ = 2.61 |
| 72-1(c) | Fish Toxicity Rainbow Trout | LC ₅₀ = 1.2 |
| 72-2(a) | Invertebrate Toxicity, Daphnid | 48-hr LC ₅₀ = 4.7 ppb |

| Table 5. Environmental Fate properties of metofluthrin. | | |
|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| PARAMETER | VALUE(S) | SOURCE |
| Solubility in water (20 °C) | 0.50 mg/L (<i>Z-isomer</i>) 0.67 mg/L (<i>E-isomer</i>) | MRID 46406754 |
| Vapor Pressure (20 °C) | 1.47 x 10 ⁻⁵ mmHg | MRID 46402005 |
| Henry's Law Constant (20 °C) | 1.5 x 10 ⁻⁵ atm m ³ /mol (<i>Z-isomer</i>) 1.1 x 10 ⁻⁵ atm m ³ /mol (<i>E-isomer</i>) | Estimated from vapor pressure & solubility ¹ |
| Hydrolysis Half-life (25 °C) | <i>pH 4 & 7: Stable</i> <i>pH 9: 33 days</i> | MRID 46406750 |
| Aqueous Photolysis Half-life (pH 4) | 6 days | MRID 46406754 (Based on 12-hour light/12-hour dark cycle with Xe lamp) |
| Aerobic Soil Metabolism Half-life | <i>MS sandy loam: DT₅₀ = 3-8 days</i> <i>CA sandy loam: DT₅₀ = 1-3 days</i> | MRID 46406751 |
| Soil Partition Coefficient (K _d) | 57.5, 75.8, 85.3, 163 mL/g | MRID 46406753 (calculated, based on submitted data) |
| Organic Carbon Partition Coefficient (K _{oc}) | 3704, 4489, 5414, 7187 mL/g _{oc} | MRID 46406753 (Calculated, based on calculated K _d) |

¹ Estimated as $Hg = \text{vapor pressure (atm)} \div \text{solubility (mol/L)}$

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DISCLAIMER: The information in this Pesticide Fact Sheet is for information only and is not to be used to satisfy data requirements for pesticide registration. The information is believed to be accurate as of the date on the document.

APPENDIX I

GLOSSARY OF TERMS AND ABBREVIATIONS

| | |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ADNT | Acute delayed neurotoxicity |
| a.i. | Active Ingredient |
| aPAD | Acute Population Adjusted Dose |
| ARI | Aggregate Risk Index |
| BCF | Bioconcentration Factor |
| CAS | Chemical Abstracts Service |
| ChE | Cholinesterase |
| ChEI | Cholinesterase inhibition |
| cPAD | Chronic Population Adjusted Dose |
| %CT | Percent crop treated |
| DAT | Days after treatment |
| DEEM-FCID | Dietary Exposure Evaluation Model - Food Consumption Intake Database |
| DNA | Deoxyribonucleic acid |
| DNT | Developmental neurotoxicity |
| DIT | Developmental immunotoxicity |
| DWLOC | Drinking Water Level of Comparison. |
| EC | Emulsifiable Concentrate Formulation |
| EEC | Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem. |
| EPA | U.S. Environmental Protection Agency |
| FQPA | Food Quality Protection Act |
| GLC | Gas Liquid Chromatography |
| GLN | Guideline Number |
| LC ₅₀ | Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/l, mg/kg or ppm. |
| LD ₅₀ | Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg. |
| LOAEL | Lowest Observed Adverse Effect Level |
| LOAEC | Lowest Observed Adverse Effect Concentration |
| LOC | Level of Concern |
| LOD | Limit of Detection |
| LOQ | Limit of Quantitation |
| mg/kg/day | Milligram Per Kilogram Per Day |
| mg/L | Milligrams Per Liter |
| MOE | Margin of Exposure |

| | |
|------------|-------------------------------------------------------------------------------------------------|
| MRID | Master Record Identification (number), EPA's system of recording and tracking studies submitted |
| MTD | Maximum tolerated dose |
| NA | Not Applicable |
| NOEC | No Observable Effect Concentration |
| NOEL | No Observed Effect Level |
| NOAEL | No Observed Adverse Effect Level |
| NOAEC | No Observed Adverse Effect Concentration |
| NPDES | National Pollutant Discharge Elimination System |
| OP | Organophosphate |
| OPP | EPA Office of Pesticide Programs |
| OPPTS | EPA Office of Prevention, Pesticides and Toxic Substances |
| PAD | Population Adjusted Dose |
| PAG | Pesticide Assessment Guideline |
| PAM | Pesticide Analytical Method |
| PHED | Pesticide Handler's Exposure Data |
| PHI | Preharvest Interval |
| ppb | Parts Per Billion |
| PPE | Personal Protective Equipment |
| ppm | Parts Per Million |
| PRZM/EXAMS | Tier II Surface Water Computer Model |
| RAC | Raw Agriculture Commodity |
| RBC | Red Blood Cell |
| RED | Reregistration Eligibility Decision |
| REI | Restricted Entry Interval |
| RfD | Reference Dose |
| SCI-GROW | Tier I Ground Water Computer Model |
| SF | Safety Factor |
| TGAI | Technical Grade Active Ingredient |
| UF | Uncertainty Factor |
| µg | micrograms |
| µg/L | Micrograms Per Liter |
| µL/g | Microliter per gram |
| USDA | United States Department of Agriculture |
| WPS | Worker Protection Standard |

APPENDIX II

Citations Considered to be Part of the Data Base Supporting the Registration of Metofluthrin.

| MRID | Citation | Receipt Date |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 46406701 | Todd, R. (2004) Product Identity and Disclosure of Ingredients of S-1264 Technical Grade. Project Number: QAP/0034. Unpublished study prepared by Insect Control and Research Inc. 5 p. | 12-Nov-2004 |
| 46406702 | Suzuki, M. (2004) Description of Beginning Materials and Manufacturing Process for S-1264: Description of Formation of Impurities. Project Number: QAP/0033. Unpublished study prepared by Sumitomo Chemical Company, Ltd. 18 p. | 12-Nov-2004 |
| 46406703 | Inoue, H. (2002) Preliminary Analysis of S-1264 Technical Grade: Final Report. Project Number: 3771, QAP/0016. Unpublished study prepared by Sumitomo Chemical Company, Ltd. 38 p. | 12-Nov-2004 |
| 46406704 | Todd, R. (2004) Certification of Ingredient Limits of S-1264 Technical Grade. Project Number: QAP/0035. Unpublished study prepared by Insect Control and Research Inc. 9 p. | 12-Nov-2004 |
| 46406705 | Inoue, H. (2002) Enforcement Analytical Methods of S-1264 Technical Grade: Final Report. Project Number: 3679, QAA/0009. Unpublished study prepared by Sumitomo Chemical Company, Ltd. 106 p. | 12-Nov-2004 |
| 46406706 | Sweetapple, G.; Lentz, N. (2003) Determination of Physical-Chemical Properties of S-1264. Project Number: QAP/0020, 015682/1. Unpublished study prepared by Ricerca Biosciences, LLC. 44 p. | 12-Nov-2004 |
| 46406707 | Sweetapple, G.; Lentz, N. (2004) Determination of UV/Visible Absorption and Boiling Point of S-1264. Project Number: 015681/1, 015681. Unpublished study prepared by Ricerca Biosciences, LLC. 36 p. | 12-Nov-2004 |
| 46406708 | Walsh, K.; Lentz, N. (2003) Determination of Water Solubility - S-1264. Project Number: 015634/1, QAP/0019, 015634. Unpublished study prepared by Ricerca Biosciences, LLC. 112 p. | 12-Nov-2004 |
| 46406710 | Beckwith, R.; Lentz, N. (2003) Determination of Dissociation Constant (pKa) - S-1264. Project Number: | 12-Nov-2004 |

| MRID | Citation | Receipt Date |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| | 015635/1, QAP/0021, 015635/0. Unpublished study prepared by Ricerca Biosciences, LLC. 34 p. | |
| 46406711 | Walsh, K.; Lentz, N. (2003) Determination of n-Octanol/Water Partition Coefficient - S-1264. Project Number: 015633/1, QAP/0023, 015633/0/1. Unpublished study prepared by Ricerca Biosciences, LLC. 86 p. | 12-Nov-2004 |
| 46406712 | Inoue, H. (2004) Stability of S-1264 Technical Grade to Normal and Elevated Temperatures, Metals and Metal Ions: Final Report. Project Number: 0007, QAP/0024. Unpublished study prepared by Sumitomo Chemical Co. Ltd., Envr. Health. 15 p. | 12-Nov-2004 |
| 46406715 | Inoue, H. (2004) Storage Stability of S-1264 Technical Grade: Final Report. Project Number: 0001, QAP/0025. Unpublished study prepared by Sumitomo Chemical Co. Ltd., Envr. Health. 54 p. | 12-Nov-2004 |
| 46406716 | Inoue, H. (2004) Corrosion Characteristics of S-1264 Technical Grade: Final Report. Project Number: 0002, QAP/0026. Unpublished study prepared by Sumitomo Chemical Co., Ltd. 11 p. | 12-Nov-2004 |
| 46406717 | Todd, R. (2004) Waiver Rationale for Dielectric Breakdown Voltage Study on SumiOne (Metofluthrin). Project Number: 110804/DIEL. Unpublished study prepared by Insect Control and Research, Inc. 4 p. | 12-Nov-2004 |
| 46406718 | Lentz, N. (2004) Determination of Freezing Point, Solvent Solubility, Absorption Spectra and Autoflammability of S-1264. Project Number: QAP/0030, 016369/1, 016369/0. Unpublished study prepared by Ricerca Biosciences, LLC. 72 p. | 12-Nov-2004 |
| 46406719 | Kunimatsu, T. (2002) Acute Oral Toxicity Study of S-1264 in Rats: Final Report. Project Number: QAT/0004, 3670. Unpublished study prepared by Sumitomo Chemical Co. Ltd., Envr. Health. 30 p. | 12-Nov-2004 |
| 46406721 | Kunimatsu, T. (2002) Acute Dermal Toxicity Study of S-1264 in Rats: Final Report. Project Number: QAT/0005, 3671. Unpublished study prepared by Sumitomo Chemical Co. Ltd., Envr. Health. 23 p. | 12-Nov-2004 |
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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 4.0 Revision Date 13.03.2010

Print Date 28.11.2010

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

Product name : Transfluthrin

Product Number : 46114

Brand : Fluka

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +441747833000

Fax : +441747833313

Emergency Phone # : +44 (0)1747 833100

E-mail address : eurtechserv@sial.com

2. HAZARDS IDENTIFICATION

Classification of the substance or mixture

According to Regulation (EC) No1272/2008

Skin irritation (Category 2)

Acute aquatic toxicity (Category 1)

Chronic aquatic toxicity (Category 1)

According to European Directive 67/548/EEC as amended.

Irritating to skin. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Label elements

Pictogram



Signal word

Warning

Hazard statement(s)

H315

Causes skin irritation.

H410

Very toxic to aquatic life with long lasting effects.

Precautionary statement(s)

P273

Avoid release to the environment.

P501

Dispose of contents/container to an approved waste disposal plant.

Hazard symbol(s)

Xi

Irritant

N

Dangerous for the environment

R-phrases(s)

R38

Irritating to skin.

R50/53

Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S-phrases(s)

S36/37

Wear suitable protective clothing and gloves.

S60
S61

This material and its container must be disposed of as hazardous waste.
Avoid release to the environment. Refer to special instructions/ Safety data sheets.

Other hazards - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

Formula : C₁₅H₁₂Cl₂F₄O₂
Molecular Weight : 371.2 g/mol

| CAS-No. | EC-No. | Index-No. | Classification | Concentration |
|--------------------------------------------------------------------------------------------------|-----------|--------------|--------------------------------------------------------------------------------------|---------------|
| 2,3,5,6-Tetrafluorobenzyl trans-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylate | | | | |
| 118712-89-3 | 405-060-5 | 607-223-00-8 | Skin Irrit. 2; Aquatic Acute 1; Aquatic Chronic 1; H315, H410 Xi, N, R38 - R50/53 | - |

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage

Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Store under inert gas. Sensitive to carbon dioxide

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Handle with gloves.

Eye protection

Face shield and safety glasses

Skin and body protection

Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form crystalline

Safety data

| | |
|-------------------------------------------|----------------------------------|
| pH | no data available |
| Melting point | no data available |
| Boiling point | 135 °C at 0.09 hPa |
| Flash point | > 35.00 °C |
| Ignition temperature | no data available |
| Lower explosion limit | no data available |
| Upper explosion limit | no data available |
| Density | 1.507 g/cm ³ at 23 °C |
| Water solubility | insoluble |
| Partition coefficient: n-octanol/water | log Pow: 5.46 at 20 °C |

10. STABILITY AND REACTIVITY

Chemical stability

Stable under recommended storage conditions.

Conditions to avoid

no data available

Materials to avoid

Strong oxidizing agents

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Hydrogen chloride gas, Hydrogen fluoride

11. TOXICOLOGICAL INFORMATION**Acute toxicity**

LD50 Oral - rat - > 5,000 mg/kg

LC50 Inhalation - rat - 4 h - > 513 mg/m³

LD50 Dermal - rat - > 5,000 mg/kg

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity

no data available

Specific target organ toxicity - single exposure

no data available

Specific target organ toxicity - repeated exposure

no data available

Aspiration hazard

no data available

Potential health effects

| | |
|-------------------|--------------------------------------------------------------------|
| Inhalation | May be harmful if inhaled. May cause respiratory tract irritation. |
| Ingestion | May be harmful if swallowed. |
| Skin | May be harmful if absorbed through skin. Causes skin irritation. |
| Eyes | May cause eye irritation. |

Additional Information

RTECS: no data available

12. ECOLOGICAL INFORMATION**Toxicity**

no data available

Persistence and degradability

no data available

Bioaccumulative potential

no data available

Mobility in soil

no data available

PBT and vPvB assessment

no data available

Other adverse effects

Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

13. DISPOSAL CONSIDERATIONS**Product**

Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION**ADR/RID**

UN-Number: 3077 Class: 9

Packing group: III

Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (2,3,5,6-Tetrafluorobenzyl trans-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylate)

IMDG

UN-Number: 3077 Class: 9

Packing group: III

EMS-No: F-A, S-F

Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (2,3,5,6-Tetrafluorobenzyl trans-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylate)

Marine pollutant: No

IATA

UN-Number: 3077 Class: 9

Packing group: III

Proper shipping name: Environmentally hazardous substance, solid, n.o.s. (2,3,5,6-Tetrafluorobenzyl trans-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylate)

15. REGULATORY INFORMATION

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

16. OTHER INFORMATION**Text of H-code(s) and R-phrase(s) mentioned in Section 3**

| | |
|-----------------|--------------------------------------------------------------------------------------------------|
| Aquatic Acute | Acute aquatic toxicity |
| Aquatic Chronic | Chronic aquatic toxicity |
| H315 | Causes skin irritation. |
| H410 | Very toxic to aquatic life with long lasting effects. |
| Skin Irrit. | Skin irritation |
| N | Dangerous for the environment |
| Xi | Irritant |
| R38 | Irritating to skin. |
| R50/53 | Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. |

Further information

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

WHO SPECIFICATIONS AND EVALUATIONS

FOR PUBLIC HEALTH PESTICIDES

TRANSFLUTHRIN

2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate



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Disclaimer¹

WHO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

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Additionally, WHO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

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¹ This disclaimer applies to all specifications published by WHO.

INTRODUCTION

WHO establishes and publishes specifications* for technical material and related formulations of public health pesticides with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 2002, the development of WHO specifications follows the **New Procedure**, described in the 1st edition of Manual for Development and Use of FAO and WHO Specifications for Pesticides (2002). This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the “FAO/WHO Joint Meeting on Pesticide Specifications” (JMPS).

WHO Specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 2002 onwards the publication of WHO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the 1st edition of the “FAO/WHO Manual on Pesticide Specifications.”

Part Two: The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by WHO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the “FAO/WHO Manual on Pesticide Specifications” and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

WHO specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. WHO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Dates of publication of the earlier versions, if any, are identified in a footnote. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

* Footnote: The publications are available on the Internet under (<http://www.who.int/whopes/quality/en/>).

PART ONE

SPECIFICATIONS

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WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

TRANSFLUTHRIN

INFORMATION

ISO common name

transfluthrin

Synonyms

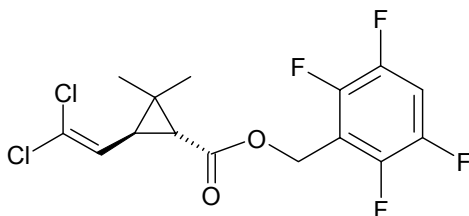
benfluthrin

Chemical names

IUPAC: 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CA: (1*R*-trans)-(2,3,5,6-tetrafluorophenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural formula



Empirical formula

C₁₅H₁₂Cl₂F₄O₂

Relative molecular mass

371.16

CAS Registry number

118712-89-3

CIPAC number

741

Identity tests

GC retention time and IR spectrum (CIPAC Handbook K, p. 121, 2003); Enantioselective GC (CIPAC Handbook L, p. 128, 2006).

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

TRANSLUTHRIN TECHNICAL MATERIAL

WHO specification 741/TC (November 2006*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in evaluation reports (741/2002 and 741/2006). It should be applicable to TC produced by this manufacturer but it is not an endorsement of it, nor a guarantee that it complies with the specification. The specification may not be appropriate for TC produced by other manufacturers. The evaluation reports 741/2002 and 741/2006, as PART TWO, form an integral part of this publication.

1 Description

The material shall consist of transfluthrin, together with related manufacturing impurities, and shall be a white to cream coloured crystalline powder, free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 Identity tests (741/TC/(M)/2, CIPAC Handbook K, p.121, 2003; CIPAC Handbook L, p.128, 2005)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 Transfluthrin content (741/TC/(M)/3, CIPAC Handbook K, p.121, 2003)

The transfluthrin content shall be declared (not less than 965 g/kg) and, when determined, the average measured content shall not be lower than the declared minimum content.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: <http://www.who.int/whopes/quality/en/>.

PART TWO
EVALUATION REPORTS

TRANSFLUTHRIN

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WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

TRANSFLUTHRIN

EVALUATION REPORT 741/2006

Recommendation

The Meeting recommended that the specification for transfluthrin, proposed by Bayer CropScience*, should be adopted by WHO.

Appraisal

Data in support of a specification for transfluthrin TC were evaluated by the JMPS in 2002 (evaluation report 741/2002) but, at the request of the manufacturer, the specification was not published. In 2004, following submissions of additional information, the manufacturer stated that new 5-batch analytical data would be generated to support production of the TC at a new site and requested reconsideration of the data and proposed specification by the JMPS. The new data and a revised proposed specification for transfluthrin TC were submitted in 2005-6.

The Meeting was provided with commercially confidential information on:

- (i) the comparability of data with those submitted for registration in Australia;
- (ii) the manufacturing process at the new site;
- (iii) the names, structures and methods of analysis of impurities;
- (iv) data from analysis of 5 batches and the manufacturing specification at the new site.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) confirmed that:

- (i) the new site manufacturing process described is essentially identical to that described in the data submitted for registration in Australia;
- (ii) the new site manufacturing specification for transfluthrin TC is identical to the declaration of composition provided for registration in Australia;
- (iii) the new site 5 batch analysis data provided to WHO comply with the declaration of composition provided for registration in Australia.

Material accountability in the 5-batch data from the new site was high (99.4-100.1%). One impurity had a reported limit of quantification (0.08 g/kg) above the stated manufacturing QC limit (0.02 g/kg). The impurity was non-relevant and the manufacturing specification for it was below the 1 g/kg threshold, therefore it was disregarded in considering whether or not the new manufacturing specification was within the earlier one. Nonetheless, the manufacturer explained that the impurity is monitored indirectly by determining the level of its precursor and, if the precursor is <0.02 g/kg, then the impurity is taken to be within the same limit.

* The manufacturer informed WHO that, in 2002, all Bayer AG assets related to crop protection and environmental science business, including the supporting data, were transferred to Bayer CropScience, which currently has the ownership.

The manufacturing process at the new site was identical to that at the previous site and the 5-batch data and manufacturing specification from the new site were all within the previous manufacturing specification. Thus a formal determination of equivalence by the Meeting was unnecessary.

WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

TRANSLUTHRIN

EVALUATION REPORT 741/2002¹

Explanation

The data for transfluthrin were evaluated in support of a new WHO specification.

Transfluthrin is/was under patent in Barbados until 2002; Poland, Czech Republic, Slovakia, South Korea, Libya, Syria, Lebanon, Kuwait, Sri Lanka, China, Dominican Republic and Brazil until 2003; Jordan, Pakistan and Taiwan until 2004; Colombia until 2005; Panama until 2007; Denmark, Norway, Finland, Hungary, Pakistan, Malaysia, South Africa, Nigeria, Turkey, Israel, Ireland, Thailand, South Korea, Japan, USA, Mexico, El Salvador, Argentina, Australia and New Zealand until 2008; Canada until 2010.

Transfluthrin has not been evaluated by the FAO/WHO JMPR and WHO/IPCS.

The WHO hazard classification of transfluthrin is “unlikely to present acute hazard in normal use.”

The draft specification and the supporting data were provided by Bayer AG, Leverkusen², in 2001.

Uses

Transfluthrin is a fast acting insecticide. It is used in household and hygiene products, mainly against flying insects, such as mosquitoes and flies, but also against material pests, such as moths (Pflanzenschutz Nachrichten Bayer, Special edition, 1995, Bayer AG, Leverkusen).

Identity

Common name

transfluthrin: E-ISO (published)

Synonyms

benfluthrin (Bayer), NAK 4455³

Chemical names

IUPAC: 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

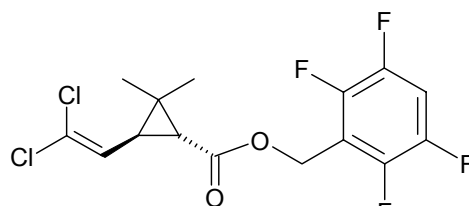
CA: (1*R-trans*)-(2,3,5,6-tetrafluorophenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

¹ 2006 footnote: minor editorial corrections were introduced in 2006, mainly to clarify the CIPAC status of the analytical method for determination of transfluthrin.

² 2006 footnote: the manufacturer informed WHO that, in 2002, all Bayer AG assets related to crop protection and environmental science business, including the supporting data, were transferred to Bayer CropScience, which currently has the ownership.

³ The development code, NAK 4455, is included because it appears in various references provided by the proposer.

Structural formula



Molecular formula

$C_{15}H_{12}Cl_2F_4O_2$

Relative molecular mass

371.2

CAS Registry number

118712-89-3

CIPAC code number

741

Identity tests

(GC retention time and IR spectrum (CIPAC Handbook K, p. 121, 2003);
Enantioselective GC (CIPAC Handbook L, p. 128, 2006))

Physico-chemical properties

Table 1. Physico-chemical properties of pure transfluthrin

| Parameter | Value(s) and conditions | Purity % | Method |
|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|----------|-----------------------------------------------------------|
| Vapour pressure | 9×10^{-4} Pa at 20°C | 97.8 | OECD 104 |
| Melting point, boiling point and/or temperature of decomposition | melting point: 32°C boiling point: 242°C decomposition temperature: sublimes at $\geq 204^\circ\text{C}$ | 98 | differential scanning calorimetry, OECD 103 |
| Solubility in water | 0.057 mg/l at 20°C | 97.8 | OECD 105 |
| Octanol/water partition coefficient | $\log K_{OW} = 5.46$ at 20°C | 97.8 | OECD 107 |
| Hydrolysis characteristics | half-life = >1 year at 25°C at pH 5 and pH 7 half-life = 14 days at 25°C at pH 9 | min. 94 | according to EPA Guideline, Subdivision N, § 161-1 (1982) |
| Photolysis characteristics | hardly affected by direct photo-degradation but accessible to natural photochemical degradation, through radical-induced oxidation | 97.8 | not stated |
| Dissociation characteristics | does not show basic or acidic properties in water | 98.4 | OECD 112, titration method |

Table 2. Chemical composition and properties of transfluthrin technical material (TC)

| | |
|-------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data | Confidential information supplied and held on file by WHO. Mass balances were 99.2 to 99.8%. |
| Declared minimum [a.i.] content | 950 g/kg |
| Relevant impurities ≥ 1 g/kg and maximum limits for them | none |
| Relevant impurities < 1 g/kg and maximum limits for them: | none |
| Stabilisers or other additives and maximum limits for them: | none |
| Melting or boiling temperature range | 32°C melting point, 242°C boiling point |

Toxicological summaries

Notes.

- (i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary below were derived from transfluthrin having impurity profiles to those referred to in the table above.
- (ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.
- (iii) A summary and references were provided by the proposer. Original reports were not submitted.
- (iv) The UK evaluation of transfluthrin (ACP 1997) was considered as part of this evaluation.

Table 3. Toxicology profile of transfluthrin technical material, based on acute toxicity, irritation and sensitization.

| Species | Test | Duration and conditions or guideline adopted | Result | Reference |
|------------|--------------------|----------------------------------------------|-----------------------------------------|-----------|
| Rat m/f | Oral | Acute, OECD 401 | LD ₅₀ > 5000 mg/kg bw | 17160 |
| Mouse m/f | Oral | Acute, OECD 401 | LD ₅₀ = 583-688 mg/kg bw | 17156 |
| Rat m/f | Dermal | Acute, OECD 402 | LD ₅₀ >5000 mg/kg bw | 17155 |
| Mouse m/f | Dermal | Acute, OECD 402 | LD ₅₀ \geq 4000 mg/kg bw | 28471 |
| Rat m/f | Inhalation | Acute, OECD 403 | LC ₅₀ >513 mg/m ³ | 17216 |
| Rabbit | Skin irritation | 4 hours, occlusive, OECD 404 | Not irritating | 15804 |
| Rabbit | Eye irritation | 24 hours, OECD 405 | Not irritating | 15804 |
| Guinea pig | Skin sensitization | Semi-occlusive, OECD 406 (Buehler Test) | Not sensitizing | 17920 |
| Guinea pig | Skin sensitization | Semi-occlusive, OECD 406 (M&K) | Not sensitizing | 17964 |

Transfluthrin is of low acute toxicity in the rat, with an LD₅₀ of >5000 mg/kg bw via each route of administration and with an acute and dermal NOEL of 100 mg/kg bw/d. The 4 h LC₅₀ was >513 mg/m³ air for male and female rats. The only sign noted during the 14 d observation period was a slight tremor in females for 5 minutes after dosing. Transfluthrin is not a skin or eye irritant, nor a skin sensitizer.

Table 4. Toxicology profile of transfluthrin technical material based on repeated administration (sub-acute to chronic).

| Species | Test | Duration and conditions or guideline adopted | Result | Reference |
|------------|--------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Rat m/f | Sub-acute oral | Sub-acute, 28 days, OECD 407 0-10-50-250 mg/kg | NOEL = 50 mg/kg bw/d | 19187 |
| Rabbit m/f | Sub-acute dermal | Sub-acute, 15 days, OECD 410 0-20-200-2000 mg/kg | NOEL = 1000 mg/kg bw/d | 19236 |
| Rat m/f | Sub-acute inhalation | Sub-acute, 4 weeks, OECD 412 0-1.6-6.6-36.6-168.1 mg/m ³ air (6 h/d; 5 d/wk) | NOEL = 36.6 mg/m ³ (\approx 13 mg/kg bw/d) | 17588 |
| Dog m/f | Sub-chronic oral diet | Sub-chronic, 13 weeks, OECD 409 0-50-350-2500 ppm | NOEL = 50 ppm (\approx 1.9 mg/kg bw/d) | R4723 |
| Rat m/f | Sub-chronic oral diet | Sub-chronic, 13-18 weeks 0-10-50-500-5000 ppm | NOEL = 50 ppm (\approx 3.5 mg/kg bw/d) | 19756 |
| Rat m/f | Sub-chronic inhalation | Sub-chronic, 90 days 0-4.9-46.7-220.2 mg/m ³ air (6 h/d; 5 d/wk) | LOEL = 46.7 mg/m ³ (\approx 17 mg/kg bw/d) | 18417 |
| Dog m/f | Chronic oral diet | Chronic, 52 weeks, OECD 452 0-30-300-3,000 ppm | NOEL < 30ppm (\approx 0.75 mg/kg bw/d) | 22638 |
| Dog m/f | Chronic oral diet | Chronic, 53 weeks, OECD 452. 0-10 ppm | NOEL = 10ppm (\approx 0.25 mg/kg bw/d) | 22678 |
| Rat m/f | Carcinogenicity and Chronic toxicity diet | Chronic, 2 years, OECD 453 0-20-200-2,000 ppm | NOEL = 20 ppm (\approx 1,0 mg/kg) NOEL for carcinogenicity = 200 ppm (\approx 9.9 mg/kg bw/d) | 22375 |
| Mouse m/f | Carcinogenicity and chronic toxicity diet | Oral feed, 2 years, OECD 451. 10, 100, and 1000 ppm diet, i.e. 2, 20, and 200 mg/kg bw/d for males, 3, 33 and 280 mg/kg bw/d for females | Males: NOAEL = 100 ppm (\approx 20mg/kg bw/d) Females: NOEL could not be determined as clinical changes were observed at the lowest dose level. Liver adenomas were observed in females at 1000 ppm dose level | 22744 |
| Rat m/f | Multi-generation study oral diet | Oral diet, 84 days, OECD 416 0-20-200-1000ppm | NOAEL = 220ppm Parental NOAEL = 200ppm (= 9 to 38 mg/kg) Neonatal NOAEL = 1,000ppm (= 50 mg/kg calculated) Reproductive NOAEL = 1,000 ppm (= 45 to 191 mg/kg) | R5352 |
| Rat f | Developmental toxicity, gavage | 10 days 0-25-55-125 mg/kg/d | Maternal NOAEL = 25mg/kg bw/d Developmental NOAEL = 125mg/kg bw/d | MTD0058 |
| Rabbit f | Developmental toxicity, oral feed [gavage] | 13 days 0-15-50-150 mg/kg/d | Maternal NOAEL = 15mg/kg bw/d Developmental NOAEL = 150 mg/kg bw/d | 18069 |

In the rat, mortalities and body tremors were seen at 250 mg/kg/d following gavage dosing. There were no mortalities following dietary administration of up 5000 ppm (approximately 40 mg/kg bw/d).

A low incidence of urinary bladder papillomas/carcinomas was observed in rats at a dietary level of 2000 ppm of transfluthrin¹. In female mice, an increased incidence of liver adenomas, but not of carcinomas, was observed at 1000 ppm, the highest dose level tested. In 2-stage studies on promoting effects in rat liver cells with diethylnitrosamine as the initiator, transfluthrin had no initiating activity but was a weak promotor (22888). Transfluthrin did not induce hepatocyte proliferation or increase mitoses in the liver *in vivo* (R5555).

Developmental studies in both the rat and rabbit provided no evidence of teratogenicity when transfluthrin was administered at doses up to 125 and 150 mg/kg bw/d, respectively. NOELs of 25 and 15 mg/kg bw/d were established for maternal toxicity in the rat and rabbit respectively.

In a dietary multi-generation reproductive toxicity study in the rat, there was no evidence of teratogenicity, foetotoxicity or reproductive toxicity in rats administered transfluthrin at doses up to 191 mg/kg bw/d. NOELs of 45 to 191 and 9 to 38 mg/kg bw/d were established for reproductive and parental toxicity, respectively.

Table 5. Mutagenicity profile of the transfluthrin technical material based on *in vitro* and *in vivo* tests.

| Test system | Test object | Concentration | Purity | Results | Reference |
|-------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------|--------------|----------|-----------|
| <i>In vitro, point mutation assays</i> | | | | | |
| <i>Salmonella</i> microsome test | <i>S. typhimurium</i> (TA 98, TA 100, TA 1535, TA 1537) | 20 to 12500 µg/plate, with and without S9 activation | 96.0% | negative | 15144 |
| <i>Salmonella</i> microsome test | <i>S. typhimurium</i> (TA 98, TA 100, TA 1535, TA 1537) | 20 to 12500 µg/plate, with and without S9 activation | 94.5% | negative | 16084 |
| HPRT-test | Chinese hamster ovary (CHO) cells | 25 to 100 µg/ml, with and without S9 activation | 94.8% | negative | 18148 |
| mitotic recombination assay | <i>Saccharomyces cerevisiae</i> D7 | 625 to 10000 µg/ml, with and without S9 activation | 94.5% | negative | 16083 |
| <i>In vitro, DNA damage assays</i> | | | | | |
| unscheduled DNA synthesis | primary rat hepatocytes | 1 to 500 µg/ml | 94.9% | negative | 21313 |
| sister chromatid exchange | Chinese hamster ovary (CHO) cells | 0.0667 to 2000 µg/ml with and without S9 activation | 94.8% | negative | R4718 |
| <i>In vivo, DNA damage assays</i> | | | | | |
| unscheduled DNA synthesis | mouse BOR:CFW1 hepatocytes | 780 and 5580 mg/kg body weight | 95.0% | negative | R3658 |
| <i>In vitro, chromosomal damage/aberration assays</i> | | | | | |
| cytogenetic study | human lymphocytes | 50 to 200 µg/ml, with and without S9 activation | 94.8%, 95.0% | negative | 18742 |
| <i>In vivo, chromosomal damage/aberration assays</i> | | | | | |

¹ The proposer noted that the effect was most likely attributable to a non-genotoxic mechanism of chronic urothelial irritation and regeneration, induced by transfluthrin or one of its metabolites (Cohen & Ellwein 1990; Bayer 1999).

| Test system | Test object | Concentration | Purity | Results | Reference |
|------------------------------------------------------------------------|------------------------------------------------------------------|---------------------------------------|--------|----------|-----------|
| micronucleus test | male and female NMRI-mouse bone marrow cells | 375 mg/kg body weight | 95.0% | negative | 16912 |
| ³² P-post-labelling assay for detection of adduct formation | male and female Wistar-rat hepatocytes and urinary bladder cells | 7 x 100 and 7 x 250 mg/kg body weight | 94.7% | negative | R6335 |

Transfluthrin was not mutagenic *in vitro* in bacteria, yeast or mammalian cells with or without metabolic activation, neither was there any evidence of mutagenicity from *in vivo* tests on rats and mice.

Table 6. Ecotoxicology profile of transfluthrin technical material.

| Species | Test | Duration and conditions | Result | Reference |
|-----------------------------------------------|---------------------------------------|-------------------------|----------------------------------------------------|--------------|
| <i>Colinus virginianus</i> (bobwhite quail) | Acute toxicity | 14 days, OECD 401 | LD ₅₀ > 2000 mg/kg NOEL = 2000 mg/kg | VB-003 |
| <i>Serinus canarius</i> (Canary bird) | Acute toxicity | 14 days, OECD 401 | LD ₅₀ > 2000 mg/kg NOEL = 2000 mg/kg | VK315 |
| <i>Salmo gairdneri</i> (rainbow trout) | Acute (flow through conditions) | 96 hours, OECD 203 | LC ₅₀ = 0.7 µg/l NOEC = 0.5 µg/l * | FF-220 |
| <i>Leuciscus idus melanotus</i> (golden orfe) | Acute (flow through conditions) | 96 hours, OECD 203 | LC ₅₀ = 1.25 µg/l NOEC = 0.89 µg/l | F0-1108 |
| <i>Daphnia magna</i> (water flea) | Acute toxicity | 48 hours, OECD 202 | EC ₅₀ = 1.2 µg/l NOEC = 0.33 µg/l | 1091 A/01 D |
| <i>Scenedesmus subspicatus</i> (green alga) | Growth inhibition | 72 hours, OECD 201 | EC ₅₀ > 0.044mg/l NOEC = 0.017 mg/l | 1091 A/01 AI |
| <i>Eisenia foetida</i> (earthworm) | Acute toxicity | 14 days, OECD 207 | LC ₅₀ = 194 mg/kg NOEC = 32 mg/kg | HBf/RG15 2 |
| Activated sludge | Microbial respiration rate inhibition | 3 hours, OECD 209 | EC ₅₀ = 10 000 mg/l | 1091 A/01 B |

* It was unclear why the difference between LC₅₀ and NOEC values was so small.

Environmental fate and behaviour

Tests of hydrolysis for transfluthrin at 25°C for 36 d gave a half-life of 14 d at pH 9 and >1 year at pH 7 and 5. Under the test conditions transfluthrin did not readily hydrolyse and, considering the very low water solubility and strong adsorption characteristics of the compound, hydrolysis is expected to play a minor role in the degradation of transfluthrin in the environment.

Transfluthrin underwent photolysis when irradiated with light of wavelengths > 290 nm with an extrapolated half-life of 17 h¹. A calculation to determine the rate of degradation of transfluthrin in air estimated the half-life to be 4.1 d.

¹ The UV absorption spectrum of transfluthrin indicates that direct photodegradation should not occur. Indirect photodegradation, by radicals generated coincidentally in the surrounding medium, was responsible for an extrapolated half-life of 17 h. In a more recent study, the half-life of indirect photodegradation was determined as 26 h (3467).

Hazard summary

Environmental toxicity tests showed that transfluthrin is of low toxicity to algae, earthworms and birds but is highly toxic to fish and daphnia. If classified using the criteria laid out in the Globally Harmonized System for classification and labelling of chemicals (UN, 2003), transfluthrin would be classified in the category Acute I, in its lower band.

Transfluthrin has not been evaluated by the WHO IPCS but the IPCS hazard classification based on acute toxicity of transfluthrin is "*unlikely to present acute hazard in normal use*" (WHO, 2002).

The FAO/WHO JMPR has not evaluated transfluthrin but the UK evaluation of the compound (ACP, 1997) was considered as part of this evaluation. The Australian Therapeutic Goods Administration of the Commonwealth Department of Health and Ageing has set an ADI of 0 to 0.003 mg/kg/d, based on the NOEL of 0.25 mg/kg bw/d for chronic dietary intake by dogs (TGA 2001).

Formulations

The main formulation types available are mosquito coils (MC) and liquid vaporizers (LV), which are registered and sold in many countries throughout the world.

Methods of analysis and testing

The analytical methods for determination of transfluthrin (including identity tests) in the TC, SL and LV are full CIPAC methods (CIPAC 2003, CIPAC 2006). Transfluthrin is determined by capillary gas chromatography with internal standardization (dipentylphthalate) and flame ionization detection.

Test methods for determination of the physical-chemical properties of technical active ingredient were mainly OECD.

Physical properties

The limits proposed for physical properties (acidity and alkalinity) of the technical material and the methods for testing them comply with the requirements of the FAO/WHO Manual (FAO/WHO, 2002).

Containers and packaging

The technical active may be stored in glass containers, plastic containers or steel drums with appropriate plastic bags.

Expression of the active ingredient

The active ingredient content is expressed as transfluthrin in g/kg.

Appraisal

There is currently no WHO specification for transfluthrin and this was a new application by Bayer AG, Leverkusen.

Transfluthrin is a synthetic pyrethroid insecticide used in household and hygiene products, mainly for the control of flying insects such as mosquitoes and flies. It has been approved for use in about 50 countries worldwide. The main formulation types

available are mosquito coils and aerosols. Evaluation of specifications for public health use was restricted to the TC.

Transfluthrin is of low acute and dermal toxicity and is classified as unlikely to present acute toxicity in normal use by the IPCS. It is not a skin or eye irritant, nor a skin sensitizer.

In a dietary multi-generation reproductive toxicity study in the rat, there was no evidence of teratogenicity, foetotoxicity or reproductive toxicity in rats administered transfluthrin at doses up to 191 mg/kg bw/d.

Transfluthrin induced a low frequency of urinary bladder adenomas/carcinomas in rats at high doses – the NOEL for non-cancer endpoints was 20 ppm, for cancer, 200 ppm, and the urinary tumours were observed at a level of 2000 ppm diet. It also induced adenomas in female mice at a high dose level. Transfluthrin had no initiating activity, but was a weak promotor of carcinogenicity. Transfluthrin was consistently negative in mutagenicity studies *in vitro* and *in vivo*; it is concluded that the tumours induced at high dose in rats and female mice are probably not produced by a genotoxic mechanism. Field and laboratory tests showed that transfluthrin is of low toxicity to algae, birds and earthworms but it is highly toxic to fish and aquatic invertebrates such as daphnia.

If classified according to the Globally Harmonized System for classification and labelling of chemicals, transfluthrin would be classified in category Acute I, lower band.

The FAO/WHO JMPR has not evaluated transfluthrin. However, the Australian authorities have set an ADI of 0 to 0.003 mg/kg bw/d (TGA 2001).

The meeting considered the issue of relevant impurities. WHO/PCS noted that the toxicity studies were all performed using transfluthrin with "similar" impurity profiles and the results showed not only a generally low toxicity but also the absence of unexpected effects. Information provided by the proposer indicated that, at the levels found in the 5 batch analysis, none of the impurities is likely to be associated with important toxic effects. WHO/PCS therefore concluded that none of the impurities was relevant and the meeting concurred with this view.

There were some minor differences in the declared composition of the technical material submitted for registration in the UK and that submitted to the WHO, in that the batch analysis data and manufacturing limits submitted to WHO indicated somewhat lower concentrations of certain impurities. The proposer explained that these were due to improvements in the quality of raw materials used and manufacturing improvements, made as part of the transition from pilot-scale to large-scale production.

CIPAC has adopted the analytical method for determination of the active ingredient in the technical material (including identity tests based on diastereoisomer ratio and stereoisomer ratios and infra-red spectroscopy) and in SL and LV formulations, which renders it acceptable for support of the specification for the TC. Transfluthrin is determined by capillary gas chromatography with internal standardization. The proposer has verified that the analytical method is capable of separation of the diastereoisomers of transfluthrin, i.e. that the corresponding *cis*-isomers would be separated and detected if present and would not be included in the measurement of transfluthrin (CIPAC, 2003).

Recommendations

The meeting recommended that the proposed specification for the technical material should be adopted by WHO¹.

References

| Bayer document number or other reference | Year and title of report or publication details |
|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
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¹ In 2004, following submissions of additional information and stating that new 5-batch analytical data would be generated to support production of the TC at a new site, the manufacturer requested reconsideration of the data and specification by the JMPS. Therefore the specification recommended for adoption in 2002 was not published.

| Bayer document number or other reference | Year and title of report or publication details |
|------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
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